EVALUATION OF SESAME GENOTYPES FOR TOLERANCE TO WATERLOGGING AND DEVELOPMENT OF MITIGATION STRATEGIES

By

SREEPRIYA S

(2017-21-020)

DEPARTMENT OF PLANT PHYSIOLOGY

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2021

Evaluation of sesame genotypes for tolerance to waterlogging and development of mitigation strategies

By

SREEPRIYA S

(2017-21-020)

THESIS

Submitted in partial fulfillment of the requirement for the degree of

Doctor of Philosophy in Agriculture

(Plant Physiology)

Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF PLANT PHYSIOLOGY

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2021

DECLARATION

I hereby declare that the thesis entitled **"Evaluation of sesame genotypes for tolerance to waterlogging and development of mitigation strategies"** is a bonafide record of research work done by me during the course of research and the thesis has not been previously formed the basis for the award to me any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara

Date: 18/10/2021

Sreepriya S.

(2017-21-020)

CERTIFICATE

Certified that this thesis entitled **"Evaluation of sesame genotypes for tolerance to waterlogging and development of mitigation strategies"** is a bonafide record of research work done independently by **Mrs. Sreepriya S (2017-21-020)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associate ship or fellowship to her.

18/10/21

Dr. T. Girija
(Major Advisor, Adisory Committee)
Professor and Head
Department of Plant Physiology
College of Agriculture, Vellanikkara

Vellanikkara
Date: 18/10/2021

CERTIFICATE

We, the undersigned members of the advisory committee of Mrs. **Sreepriya S (2017-21-020)**, a candidate for the degree of **Doctor of Philosophy in Agriculture**, with major field in **Plant Physiology**, agree that the thesis entitled **"Evaluation of sesame genotypes for tolerance to water logging and development of mitigation strategies"** may be submitted by **Mrs. Sreepriya S (2017-21-020)**, in partial fulfillment of the requirement for the degree.

18/10/21

Dr. T. Girija
(Chairman, Advisory committee)
Professor and Head
Department of Plant Physiology
College of Agriculture, Vellanikkara

18/10/21

Dr. Abida P. S.
(Member, Advisory Committee)
Professor and Head (CPBMB)
College of Agriculture, Vellanikkara

18/10/21

Dr. Deepu Mathew
(Member, Advisory Committee)
Associate Professor (CPBMB)
College of Agriculture, Vellanikkara

18.10.21

Dr. Meera V Menon
(Member, Advisory Committee)
Professor
Department of Agronomy
College of Agriculture, Vellanikkara

18/10/21

Dr. Bindu M. R.
(Member, Advisory Committee)
Professor
Department of Plant Breeding
and Genetics, FSRS,
Sadanandapuram

EXTERNAL EXAMINER

18.10.2021

Dr. G. Rama Rao

Associate Director of Research
Regional Agricultural Research Station
CHINTAPALLE-531 111
Visakhapatnam Dist. (AP)

ACKNOWLEDGEMENT

First and foremost I bow my head before the ***Almighty God*** *who enabled me to successfully complete the thesis work.*

It is with great respect I avail this opportunity to express my deep sense of gratitude and indebtedness to my major advisor, ***Dr. T. Girija,*** *Professor and Head (Retd.), Department of Plant Physiology, College of Agriculture, Vellanikkara for her meaningful guidance, Constructive suggestions, kind consideration, support, motherly approach and wholehearted co-operation rendered throughout the course of my study. I really consider it my greatest fortune in having her guidance for my research work and my obligation to her lasts forever.*

I express my heartfelt thanks to ***Dr. Parvathi M. S****, Assistant Professor, Department of Plant Physiology, College of Agriculture, Vellanikkara for her inspiration, valuable and timely suggestions, helpful advice, cooperation at any time with care and friendly approach.*

I think it is my privilege to express my sincere thanks to ***Dr. P. S. Abida*** *Professor and Head, Department of Plant Physiology, College of Agriculture, Vellanikkara,* ***Dr. Meera V Menon****, Professor, Department of Agronomy, College of Agriculture, Vellanikkara and* ***Dr. Bindu M. R.*** *Professor, Department of Plant Breeding and Genetics FSRS, Sadanandapuram, members of my advisory committee for their expert advice, valuable guidance and cooperation throughout the research programme.*

I sincerely thank ***Dr. Deepu Mathew,*** *Assistant Professor, CPBMB, College of Agriculture, Vellanikkara, who is also a member of the advisory committee, for his timely suggestions, critical comments and providing me all the*

facilities at molecular laboratory that helped me to successfully complete my research work.

I thankfully remember the services rendered by ***Dr. Sajeena****, Assistant Professor (Plant Pathology), IFSRS, Karamana and* ***Dr. Sharavananan Raju,*** *Principal Scientist, Division of crop production, CTCRI, TVM and* ***Dr. Gayathri G****, Assistant Professor, Department of Botany, Sree Krishna College, Guruvayur.*

I express my sincere thanks to my dear friends ***Vineetha, Amritha K. Annjoe, Neeraja chechi, Vishnu B.R, Priyanka, Vipul, Durga, Ajaysree, Apana chechi, Lishma chechi, Lalith, Nadhika, Salpriya, Ruby and Karishma*** *for their constant help and encouragement that made me confident in difficult situations.*

My heartfelt gratitude cannot be explained in words for the constant support and affection of my dearest seniors and juniors especially ***Garggi chechi, Sreelaja, Athira K.A, Linu C, Anil A. S., Femina, Mikhina, Aswathy and Akshaya*** *throughout my research work and course.*

I have infinite pleasure to express whole hearted thanks for the innumerable help and support especially from ***Sheena chechi, Bindu chechi and Arun S. Kadakkal.***

I extent my heartfelt thanks to my husband ***Mr. Rohit Murali*** *for his care, constant support and for being with me in difficult times and I express my gratitude to our* ***Father, A. Muraleedharan Nair, Sister, Dr. Sreelekshmi M. S.*** *and* ***Grandmother, Santhakumari Amma*** *for their support and care.*

I am in dearth of words to express my love towards ***my Father, Santhosh kumar D, Mother, R. Usha devi,*** *and my loving sister* ***Krishnapriya*** *for their boundless affection, moral support, eternal love, deep concern, prayers and personal sacrifices which sustains peace in my life.*

*I owe special thanks to Librarian, **Dr. Francis**, College of Agriculture and all other staff members of Library, who guided me in several ways, which immensely helped for collection of literature for writing my thesis.*

*I express my deep sense of gratitude to **Kerala** Agricultural University for financial and technical support for persuasion of my study and research work.*

It would be impossible to list out all those who have helped me in one way or another in the successful completion of this work. I once again express my heartful thanks to all those who helped me in completing this venture in time.

Sreepriya S

CONTENTS

LIST OF TABLES

LIST OF TABLES (Contd.)

LIST OF TABLES (Contd.)

LIST OF TABLES (Contd.)

LIST OF TABLES (Contd.)

LIST OF FIGURES

LIST OF FIGURES (Contd.)

LIST OF PLATES

LIST OF PLATES (Contd.)

Abbreviations

DAS	- Days After Sowing
DAE	- Days After Emergence
%	- Per cent
GOK	- Government of Kerala
NAA	- 1-naphthaleneacetic acid
PGPR	- Plant growth promoting rhizobacteria
QTL	- Quantitative trait loci
ROS	- Reactive oxygen species
CAT	- Catalase enzyme
ROS	- Reactive Oxygen Species
N	- Nitrogen
NO_3^-	- Nitrate
MDA	- Malondialdehyde
PEG	- Poly ethylene glycol
MPa	- Mega pascal
EST	- Expressed sequence tag
SA	- Salicylic acid
K	- Potassium
ppm	- Parts per million
SSR	- Simple sequence repeats
ISTA	- International Seed Testing Association
ORF	- Open reading frames
PCR	- Polymerase chain reaction
NRase	- Nitrate reductase
LDH	- Lactate dehydrogenase
ADH	- Alcohol dehydrogenase
PDC	- Pyruvate decarboxylase

Introduction

INTRODUCTION

Sesame (*Sesamum indicum* L.) is an annual erect plant belonging to the family Pedaliaceae, cultivated since antiquity for its seed, oil and flavorsome value. It is also known as gingelly, til, benne seed and popularly known as "Queen of oilseeds" due to its high degree of resistance to oxidation and rancidity (Bedigian and Harlan, 1986). Sesame seed contains 50-60% of high quality oil which is rich in polyunsaturated fatty acids (PUFA) and natural antioxidants, sesamin, sesamolin and tocopherols (Brar and Ahuja, 1979). These bioactive components enhance the stability and keeping quality of oil and also have health benefits. Sesame seed is high in protein, vitamin B1, dietary fiber and is an excellent source of phosphorus, iron, magnesium, calcium, manganese, copper and zinc. Moreover, it also contain two unique substances, sesamin and sesamolin, which belong to a group of special beneficial fibers called lignans and have a cholesterol lowering effect in humans and prevent high blood pressure and increase vitamin E supplies in animals.

Although it is an ancient crop with high economic value, sesame is listed as a neglected and underutilized crop by the International Plant Genetic Resources Institute (IPGRI) due to its limited cultivation and expansion in the world (Dossa, 2016).

India have unique distinction as a producer, consumer, exporter and importer of sesame. India exports about 300,000 tons of sesame seed, accounting for approximately 15per cent of the global sesame trade. The country ranks second in area and production of sesame (FAOSTAT, 2020). However, in Kerala, the crop provides only a small contribution to the total area of oil seeds. From 2001-2002 to 2018-2019, 57 % reduction in area and 44 % reduction in production of sesame is reported (ECOSTAT, 2020). In Kerala, sesame is cultivated in summer rice fallows and rabi uplands. Moderately fertile, well drained soils with a pH ranging from 5.5 to 8.0 is best for the crop.

The changes in rain fall pattern of the state has affected sesame production. Waterlogging is a common adverse environmental condition that limits plant growth. Due to the erratic pattern of rainfall and other extreme climate events, waterlogging

currently is a major threat to crop production worldwide. Like other stresses, the effects of waterlogging are intricate, as the magnitude of stress varies with plant species, temperature, soil condition, plant age, sunlight, stress duration *etc* (Setter *et al*., 2009). When grown on soils with poor drainage, sesame is adversely affected and can suffer yield losses of more than 30% (in severe cases, 50–90%) (Snowden and Wheeler, 1993). Waterlogging in sesame causes premature senescence due to leaf chlorosis, necrosis, defoliation, and reduced nitrogen fixation, leading to cessation of growth and reduced yields (Wei *et al*., 2013).

Studies on water logging resistance of different genotypes is limited. Therefore, selecting water logging resistant germplasm is important for popularizing the cultivation of this crop and also for developing tolerant varieties by the sesame breeders. Dossa *et al*. (2017) had foreseen demand for highly resistant plants mingled with desired agronomical traits as a future requirement for promoting sesame cultivation.

As unpredictable excessive rainfall cannot be prevented, waterproofing sesame is an urgent need. Mitigating the negative effect of waterlogging stress with ameliorants will be a beneficial strategy for reducing the impact of water logging stress on the crop. Nutrient deficiency is a problem associated with waterlogging. The uptake of Nitrogen (N), Phosphorus (P), Potassium (K) and Calcium (Ca) are reported to be affected under waterlogging (Gutierrez Boem *et al*., 1996). Strategic use of fertilizers prior to the stress may alleviate nutrient deficiency. Moreover waterlogging damages is reported to be alleviated by applying suitable plant growth regulators at appropriate growth stage (Nguyen *et al*., 2018a). Foliar application of Salicylic acid, Tricyclazole and 1-naphthaleneacetic acid (1-NAA) were reported to be promoting the plant response to different environmental stresses (Habibzadeh, 2012; Xing *et al*., 2016; Shakirova *et al*., 2003). Seed inoculation and foliar spray of biofertilizers such as plant growth-promoting rhizobacteria (PGPR), have been used to reduce the negative effects of stress conditions in plants (Wu *et al*., 2005; Saleem *et al*., 2007).

Even though sesame is regarded as a drought tolerant crop, it is sensitive to drought at different developmental stages such as germination (Bahrami *et al*., 2012; Mensah *et al*., 2006) and seedling growth (Boureima *et al*., 2011). Drought responses of sesame are reported to be genotype specific (Hassanzadeh *et al*., 2009a, Kadkhodaie *et al*., 2014). To identify the climate resilient sesame genotypes, comparing the tolerance ability of sesame genotypes to both stresses (waterlogging and drought) will be advantageous.

Improvement of sesame by modern technology such as genetic and genomic tools has been lagging behind other model plants (Dossa, 2016). Identification of molecular markers linked with the major genes or QTL contributing to quantitative trait will help to speed up molecular breeding for waterlogging tolerance in sesame. Confirmation of involvement of reported genes and markers will be necessary for marker assisted selection in the native population.

In this context, the experiment has been envisaged with the objectives:

1. To identify the constraints of sesame farmers in Kerala

2. Screening selected sesame genotypes for tolerance to waterlogging stress

3. Study the effect of waterlogging on morpho-physiological parameters of sesame

4. Waterproofing sesame by mitigating the growth inhibitory effect of waterlogging

5. Screening the same sesame genotypes for tolerance to drought at seed germination stage

6. To study the impact of drought stress on seedling parameters of seleced sesame genotypes

7. Molecular characterization of selected sesame genotypes by the identification of candidate genes for waterlogging stress

Review of literature

2. REVIEW OF LITERATURE

2.1 FARMER SURVEY FOR CONSTRAINT ANALYSIS

Sesame is an ancient oilseed crop of India and also an important agricultural export commodity. India ranks second in area and production of sesame (FAOSTAT, 2020). Since 2014, Indian sesame seed production was down by 1.8 per cent year by year. The Indian crop of sesame production was short by 60 per cent in 2018, as against 418000 MT in 2017 (Anon., 2019). At the end of fiscal year 2019, India produced 0.69 million metric tons of sesame. This was similar to the previous fiscal year (Statista, 2020). Uttar Pradesh, Rajasthan, Madhya Pradesh, Andhra Pradesh, Maharashtra, Gujarat, Tamil Nadu, Orissa and Karnataka are the major sesame growing states, whereas, Kerala is one of the lowest sesame producing states (Sharma, 2014).

2.1.1 SCENARIO OF SESAME PRODUCTION IN KERALA

Sesame provides only a small contribution to the total area of oil seeds in Kerala. Sesame occupied only 378 ha of area in Kerala during the year 2018-19 with a production of 158 MT (GOK, 2020a). During the period from1993-94 to 2009-2010, the area under sesame cultivation in Kerala recorded a decline of 3.5 thousand ha. The GOK, report of 2016 showed 56.30 per cent decrease in area and 58 per cent decrease in production of sesame in Kerala during the period from 2005-2006 to 2014-15. On analyzing the area of the past last 10 years, sesame cultivation was the highest during agricultural year 2009-10 when the area was 608 ha. Area under sesame production declined about 80 % during the period 2001-2002 to 2017-2018 (878 ha to 239 ha) leading to a decrease of 79 % production (GOK, 2016). From 2001-2002 to 2018-2019, 57 per cent reduction in area and 44 % reduction in production of sesame is reported (ECOSTAT, 2020).

Cultivation of sesame is restricted to a few districts and the decrease in area of the crop is observed in all these districts with around 94 per cent decline in area (GOK, 2005). Among the sesame cultivating districts, Alappuzha district has 1st position in area with 45.63 per cent of the total cultivated area (GOK, 2014)

contributing to 43 per cent of total sesame production followed by Kollam district with 36 per cent. Thrissur district is reported to have the highest productivity of sesame (GOK, 2020b). In Kerala, sesame is cultivated as a summer crop while the leading sesame producers of India, cultivate it as a kharif crop (IOPEPC, 2017). As per the government reports, the cultivation of sesame may not be seen in the state within a few years.

2.1.2 CHALLENGES IN SESAME PRODUCTION

Sesame thrives well in a harsh environment and requires limited fertilizer, water, without the need for pesticides due to high levels of natural tolerance for diseases and insects. However, yield is highly variable and depends on growing environment, cultural practices, and cultivars (Myint *et al*., 2020). According to Najeeb *et al*. (2012), for the sustainable production of the crop, there is a need for abiotic stress tolerance to be integrated in sesame. This requires identification of important stress factors that reduce sesame productivity.

According to the report of sesame village project published by MSSRF (2007), availability of quality seed materials for sowing, low yield, pest and diseases, labour availability, lack of awareness of suitable management practices to ensure good yield, high yield variation within the field, vagaries of nature and lack of good returns from sale were the constraints of sesame growers in India.

Drought and salinity are the major factors that limit sesame productivity. It is sensitive to drought mainly at the vegetative stages in all growing regions and has low production in semiarid regions due to drought stress (Boureima *et al*., 2012). Grain yield as well as oil yield and quality depend on genotypes and drought intensity.

According to Kumaraswamy *et al*. (2015) majority of sesame crop is harvested manually, hence there is a need for promoting mechanized harvesting in this crop to make its cultivation economically viable.

According to a survey conducted in West Africa, sesame marketing was the most important impediment. The problem is basically lack of reliable markets for the stock (Dossa *et al*., 2017).

Lack of fast-adapting cultivars, capsule shattering, uneven ripening, poor crop stand establishment, lower fertilizer responses, profuse branching, low harvest index, indeterminate growth habit, and susceptibility to diseases are the limiting factors in sesame production worldwide (Tripathy *et al*., 2019).

Sesame is sensitive to waterlogging, salinity, and chilling that limit sustainable production (Tripathy *et al*., 2019). Sesame growth and yield decreases after 2–3 days of waterlogging when the crop is grown on poorly drained soils. Waterlogging significantly reduces plant growth, leaf axils per plant, seed yield, and net photosynthesis (Ucan *et al*., 2007).

2.2 IMPACT OF WATERLOGGING ON SESAME GENOTYPES

2.2.1 Impact of waterlogging on morphological characters

2.2.1.1 *Survival percentage*

Several studies reported that survival rate under hypoxic conditions is an important mean to assess the degree of flooding tolerance (Xu and Mackill, 1995; Nandi *et al*., 1997; Martin *et al*., 2006; Hussain *et al*., 2014). According to Parelle *et al*. (2010), the advantage of measuring survival rate is that variability in this trait is directly related with genetic variability for flooding tolerance. Hassan *et al*. (2001) recorded 35 per cent mortality of summer grown sesame plants subjected to 48hr of waterlogging at crop establishment period (0-10 DAE). Athul (2016), observed highest survival of 97.60 per cent in *Sesamum malabaricum* (wild sesame) plants subjected to waterlogging for 72 hr at 20DAS whereas the variety SV2 recorded the least survival (55.20 %) under field condition.

2.2.1.2 *Plant height*

Hassan *et al*. (2001) reported 38 per cent reduction in plant height when sesame plants were imposed with waterlogging for 48 hr at vegetative stage. Hossain

(2006) reported that when sesame plants were waterlogged for 24, 36 and 48 hrs, plant height reduced to 86, 78, 71 cm respectively, whereas non-waterlogged plants recorded a height of 87 cm at 45 DAS.

Sarkar *et al*. (2016) conducted an experiment with two sesame varieties *viz*., BARI Til 2 and BARI Til 3, which were waterlogged for 36 hr at vegetative and flowering stages. He observed 17.39 per cent and 32.60 per cent reduction in plant height in BARI Til2 and BARI Til 3 respectively.

Saha *et al*. (2016) observed that when different sesame genotypes namely, BD-6980, BD6985, BD-6992 and BD-7012 were waterlogged for 3 days at vegetative stage, all three showed reduced plant height compared to control during both waterlogging period and recovery period.

2.2.1.3. *Root characters*

Under anaerobic conditions, root metabolism and root growth are inhibited, as the lack of O_2 affects the energy status of the plant (Drew, 1992; Voesenek *et al*., 2003). Normal root development was replaced by adventitious roots and it eventually led to increase in number of roots compared to control (Mano and Omori, 2007). According to Satomi *et al*. (2014), root dry weight, root length and root surface area are important indices of flood tolerance.

Saha *et al*. (2016) observed a reduction in root length in all genotypes (BD-6980, BD6985, BD-6992 and BD-7012) studied during both waterlogging period and recovery period.

Athul (2016) subjected 30 sesame genotypes to 72 hr waterlogging. He observed that among the genotypes, wild sesame (*Sesamum malabaricum*), Ayali (local cultivar), Thilarani and Rama recorded highest number of undamaged roots while, TKG-22 recorded the lowest number of roots. He also observed higher root length in Ayali and Thilak varieties, while it was low in wild sesame.

2.2.1.4 *Leaf number*

Leaf number per plant of sesame was reduced from 52 (irrigated every 15 days interval) to 42 when sesame plants were waterlogged for 20 days at a height of 1 cm from soil surface at 30 DAS (Mensah *et al*., 2006).

2.2.1.5 *Shoot and root dry weight*

Mensah *et al*. (2006) examined the impact of waterlogging on shoot and root dry weight of sesame. He used 30 day old sesame plants and kept them in water logged for 20 days at a height of 1 cm from soil surface and compared it with plants irrigated at every 15 days interval. He observed a decrease in shoot and root dry weight of sesame plants from 3 g and 1.3 g to 2.5g and 1.1g respectively. According to Saha *et al*. (2016) root dry weight of different sesame genotypes were reduced to 28-81 per cent at waterlogging period and 57-69 per cent at recovery period compared to non-waterlogged plants.

2.2.2 Impact of waterlogging on photosynthetic parameters

Xu *et al*. (2012) observed a reduction in photosynthetic rate from 15.5 μmol $CO_2/m^2/s$ to 6.3 μmol $CO_2/m^2/s$ in waterlogging tolerant genotype WTG-2541, whereas in waterlogging sensitive genotype, WSG-EZhi2, recorded a reduction from 15 μmol $CO_2/m^2/s$ to 0.26 μmol $CO_2/m^2/s$. The closure of stomata and reduced stomatal conductance have been reported to cause a decrease in intercellular CO_2 concentration (Ci) and carboxylation efficiency in maize plants under waterlogged condition (Zhang and Zhang, 1994). Waterlogging results in the reduction in transpiration rate and stomatal conductance in maize (Baranwal and Singh, 2002) and lucerne (Smethurst *et al*., 2005) genotypes.

2.2.3 Impact of waterlogging on enzyme activity in root

As reported by Fukao and Bailey-Serres (2004) plants respond to hypoxia by generating metabolic energy from fermentative glycolysis rather than from oxidative respiration. Two main fermentation pathways that are active in plants during flooding are ethanol and lactic acid fermentation (Dennis *et al*., 2000). Alcohol

dehydrogenase and lactate dehydrogenase are the two important anerobic proteins involved in these fermentation pathways.

Wei *et al*. (2013) conducted a waterlogging experiment with one waterlogging tolerant (ZZM2541) and one sensitive genotype (Ezhi-2) of sesame and observed that during 2, 4, 6 and 8 days of waterlogging, there was an increase in alcohol dehydrogenase activity in ZZM2541 which was more pronounced than in Ezhi-2, while lactate dehydrogenase activity was greater in Ezhi-2.

2.2.4 Impact of waterlogging on biochemical parameters

2.2.4.1. *Catalase enzyme activity*

Root hypoxia causes photoxidative damage to leaves by increased generation of reactive oxygen species (ROS) superoxide ($O2^-$), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH•), which readily attack leaf chloroplasts and lead to leaf chlorosis and senescence. The ROS scavenging is carried out by a number of well-characterized enzymes such as catalase (CAT). It has been reported that high levels of catalase activity is critical for survival of sesame (Wei *et al*., 2013), rice (Ushimaro *et al*., 1992), tobacco (Hurng and Kao, 1994), sweet potato (Hwang *et al*., 1999), mungbean (Ahmed *et al*., 2002), sunflower (Grassini *et al*., 2007) and wheat (Li *et al*., 2011) under water logged conditions.

Xu *et al*. (2012) observed that under flooding stress for 48 hr at 3 leaf stage, catalase activities of sesame genotypes, WTG-2541(tolerant) and WTG-2413 (tolerant) decreased by 60.90 and 35.28 per cent, respectively. By contrast, the CAT activity of WSG-EZhi2 (sensitive) decreased by 78.78 per cent, indicating less effect of flooding stress on tolerant genotypes compared to the susceptible one.

According to Anee *et al*. (2019) there was 9 per cent, 10 per cent, 23 per cent, and 33 per cent decline in catalase enzyme activity at 2, 4, 6, and 8 days of waterlogging, respectively with respect to control in *Sesamum indicum* L. cv. BARI Til-4 at vegetative stage. Catalase activities increased under waterlogged sesame plants in the following genotypes BD-6980, BD-6985, BD-6992 and BD-7012) under waterlogged condition and during recovery period while for BD 6985 it was lower during recovery period (Saha *et al*., 2016).

2.2.4.2 ***Nitrate reductase enzyme activity***

Nitrate reductase is a key enzymes in nitrogen metabolism, whose activities have been used as representative biochemical markers to evaluate N status in plants (Ren *et al*., 2017a). Reduction of nitrate reductase activity in leaves of waterlogged plants is due to a rapid depletion of NO_3^- and oxygen under anaerobic conditions (Sung and Sun, 1990; Hoff *et al*., 1992).

2.2.4.3. ***Chlorophyll content (mg g⁻¹)***

Sesame plants at 30 DAS when waterlogged for 20 days at a height of 1 cm from soil surface, showed a reduction in total chlorophyll content from 3.6 mgg^{-1} to 2.3 mgg^{-1} when compared with plants irrigated at 15 days interval (Mensah *et al*., 2006). Wei *et al*. (2013) conducted a waterlogging experiment with one waterlogging tolerant (ZZM2541) and one sensitive genotype (Ezhi-2) of sesame and pointed out that there was 82 % and 18 % reduction in chlorophyll content in Ezhi-2 and ZZM2541 respectively compared to their respective non waterlogged control. Anee *et al*. (2019) investigated the response of 2, 4, 6, and 8 days of waterlogging on *Sesamum indicum* L. cv. BARI Til-4 at vegetative stage. His study proved that as the waterlogging time progressed the chlorophyll content reduced.

2.2.4.4 ***Soluble protein content (mg g⁻¹)***

Decrease in soluble protein content in leaves of tolerant and susceptible sesame genotypes subjected to 8 days of waterlogging stress was reported by Wei *et al*. (2013). Under waterlogging stress soluble protein content increased in maize (Rai *et al*., 2004), cotton (Guo *et al*., 2010) but not in wheat (Olgun *et al*. 2008).

2.2.4.5 ***MDA (malondialdehyde) content*** **(nmol g^{-1})**

Xu *et al*. (2012) observed that under flooding stress for 48 hr, leaf MDA content increased by 7.74 and 2.10 per cent in tolerant (WTG-2541, WTG-2413) and 28.73 per cent in susceptible (Ezhi-2) sesame genotypes at 3 leaf stage. Wei *et al*. (2013) recorded 1.6 fold higher MDA content in susceptible genotype of sesame compared to tolerant ones.

Saha *et al*. (2016) reported a higher MDA content in waterlogged plants of all the four sesame genotypes studied both under waterlogged condition and also

during the recovery period (14.41 to 14.70 nmol g^{-1} FW and 10.66 to22.03 nmol g^{-1} FW, respectively) compared to control plants (9.92 to 13.7 nmol g^{-1} FW and 8.42 to 18.27 nmol g^{-1} FW). A progressive period of waterlogging for 2, 4, 6, and 8 days vegetative stage in sesame genotypes resulted in time dependent increase of MDA content of nearly 39 per cent (Anee *et al*., 2019).

2.2.5 Impact of waterlogging on root anatomy

One basic strategy to cope up with waterlogging is to improve gas exchange. Gas-filled spaces called aerenchyma are considered to be an efficient mechanism to ameliorate low oxygen stress. It facilitates gas exchange between aerial and submerged plant parts by reducing the diffusion resistance to gas exchange imposed by cells (Steffens *et al*., 2010).

It was observed that tolerant sesame genotype, ZZM2541 had developed adventitious roots and plentiful of aerenchyma in root and stem whereas in the susceptible genotype Ezhi-2, only some disorganized aerenchyma could be seen (Wei *et al*., 2013). Aerenchyma development in response to waterlogging has been reported in different crops such as rice (Steffens *et al*., 2010), barley (Zhang *et al*., 2015) and Maize (Rajhi and Mhadhbi, 2019).

2.2.6 Impact of waterlogging on yield and yield attributes

Waterlogging has been reported to have significant effect on yield attributes such as number of branches, number of capsules, number of seeds per capsule and 1000 seed weight of sesame.

Ghoi *et al*. (1996) in soyabean, Sarkar *et al*. (2016) and Athul (2016) in sesame reported reduction in number of branches due to waterlogging. Hossain and Salahuddin (2001) found that the number of capsules per plant was reduced significantly in sesame by waterlogging.

Hassan *et al*. (2001) reported 16 per cent, 71 per cent and 6.6 per cent reduction in number of capsules per plant, number of seeds per capsule and 1000

seed weight when sesame plants were waterlogged for 48 hr at vegetative stage. He observed a reduction in seed yield per plant from 4.08 g to 1.94 g.

According to Choi *et al*. (1990) and Beltrao *et al*. (1997), longer period of waterlogging decreased 1000 seed weight in sesame. In contradiction to this, Hassan *et al*. (2001) and Sarkar *et al*. (2016) reported that thousand seed weight was not affected due to waterlogging.

Sarkar *et al*. (2016) reported 42.50 per cent and 58.30 per cent reduction in number of capsules per plant in 2 sesame varieties *viz*., BARI Til 2 and BARI Til 3 respectively which were waterlogged for 36 hr. Reduction in number of seeds per capsules and reduction in yield per hectare (51 % , 58 %) were also observed.

Saha *et al*. (2016) reported 24-44 per cent reduction in seed yield per plant, 3-23 per cent reduction in number of seeds per capsules, 0-7 per cent reduction in 1000 seed weight in four sesame genotypes (BD-6980, BD- 6985, BD-6992 and BD-7012) which were waterlogged for 3 days at vegetative stage.

2.3 SCREENING FOR DROUGHT STRESS TOLERANCE

Crops are exposed to various environmental stresses during their life span and among them, drought stress has the most detrimental effect on the crop survival and productivity (Lambers *et al*., 2008; Alqudah *et al*., 2011). Sesame, owing to its high oil content, antioxidants and protein content, is a highly valued crop (Koca *et al*., 2007). Understanding drought response in the crop is important because it is regularly subjected to mild or severe water stress (Golestani and Pakniyat, 2007). Even though, sesame is considered to be relatively a drought tolerant crop, it is sensitive to drought at different developmental stages such as germination (Boureima *et al*., 2011; Bahrami *et al*., 2012, Mensah *et al*., 2006), seedling growth (Boureima *et al*., 2011; Bahrami *et al*., 2012), and latter developmental stages (Bahrami *et al*., 2012, Boureima *et al*., 2011, Mensah *et al*., 2006, Ucan and Killi, 2010). Several traits of the plant have been reported to be affected by drought stress including the germination rate, plant growth, flowering, number of capsules per

plant, seed yield, yield and quality of oil (Bahrami *et al*., 2012; Boureima *et al*., 2016; Hassanzadeh *et al*., 2009b; Kassab *et al*., 2012; Sun *et al*., 2016).

Studies also show that the drought effects and drought responses of sesame are genotype specific (Hassanzadeh *et al*., 2009a, Bahrami *et al*., 2012, Boureima *et al*., 2011, Kadkhodaie *et al*., 2014). Hence, identification of drought resistant genotypes is very important for breeding programs.

According to Juenger (2013), variation in drought tolerance is almost always found in a large collection of accessions. Sesame harbours a huge diversity that is probably linked to its cultivation in a large range of environments coupled with long-term natural and artificial selections (Bedigian and Harlan, 1986; Wei *et al*., 2015). Also, resistance to drought in sesame is important in many areas with low rainfall. Moreover, in Kerala, sesame is grown as a rainfed crop in summer.

2.3 1 Poly Ethylene Glycol (PEG) in drought stress study

In recent years, researchers have often used different osmotica to lower the waterpotential of plant growth media. This approach has many advantages, waterpotential can be controlled precisely whereas a large number of treatments can be performed quickly in a reproducible way (Verslues *et al*., 2006). Osmotica with lower molecular weight such as mannitol can freely penetrate cell wall pores and cause plasmolysis rather than cytorrhysis. Poly ethylene glycol of molecular weight 6000 (PEG-6000) or above cannot enter plant cells pore (Ranjbarfordoei *et al*., 2000). Because PEG-6000 does not enter the apoplast, water is withdrawen from the cell and cell wall (Van den Berg and Zeng, 2006) without damaging cell content. Hence, PEG can be used as a solution which can mimic dry soil conditions

2.3.2 Screening for drought tolerance at seedling stage

Seed germination and seedling development are being used in various selection procedures for identifying drought tolerant genotypes. Test usually involve seed germination in osmoticum (Helmeric and Pfeifer, 1954; Williams *et al*., 1967, Bassiri *et al*., 1977, Ashraf and Abu-Shakra, 1978) and seedling growth or survival under conditions of induced moisture deficit (Ashtox, 1948, Younis *et al*., 1963, Kilen and Andrew, 1969).

Induction of water stress during seedling stage has been adopted as a screening method in crops like wheat (Dhanda *et al*., 2004), sorghum (Gill *et al*., 2002; Bibi *et al*, 2010), maize (Mohammadkhani and Heidari, 2008; Farsiani and Ghobadi, 2009; Khayatnezhad, 2010) and sunflower (Rauf *et al*., 2008).

According to Rauf (2008) the benefits of screening genotypes at seedling stages are low cost, ease of handling, less laborious and getting rid of susceptible genotypes at the earliest. Moreover, field experiments related to water stress are difficult to handle due to drought interactions with other abiotic stresses and other environmental interferences. (Rauf, 2008).

2.3.3 Seedling parameters under study

2.3.3.1 ***Seed germination***

Heikal *et al*. (1982) and Mensah *et al*. (2006) found that low level of drought stress had not any significant effect on germination, by increasing levels of drought, germination and seedling growth reduced, on the other hand, drought stress level has negative correlation with germination and seedling growth.

Bouremia *et al*. (2011) reported that the mean germination per cent of all the 22 sesame mutants studied were reduced from 96 per cent to 76.35 per cent at an osmotic potential of -1MPa. He also reported that sesame seed germination was reduced by 22.43 per cent at -1MPa when compared to control and inhibited for all genotypes at an osmotic potential ≤1.5MPa which is close to wilting point in most sesame field conditions. Germination and seedling growth of Sri Lankan grown sesame cultivars are affected by the PEG induced water stress as reported by Dissanayake *et al*. (2019).

Germination of sunflower was inhibited in presence of polyethylene glycol - 6000, at osmotic pressure lower than -5 bars (Smok *et al*., 1993).

2.3.3.2 ***Percentage of abnormal seedlings***

Seedlings were regarded as abnormal when the radicle or plumule was deformed, damaged or decayed and which fail to grow to healthy seedlings after the test period. Pavli *et al*. (2020) recorded increase in abnormal seedlings under PEG treatment in soyabean cultivars. Badr *et al*. (2020) reported that the 15 per cent and

20 per cent PEG treatments in maize genotypes resulted in a significant proportion of abnormal seedlings.

2.3.3.3 *Root length*

Roots are the place where plants first encounter water stress, it is likely that roots may be able to sense and respond to the stress condition (Xiong *et al*., 2006; Khodarahmpour, 2011) and plays an important role in water stress tolerance by reduction in leaf expansion and promotion of root growth (Saddam *et al*., 2014). Root length at seedling stage provides a fair estimate about the root growth in field (Ali *et al*., 2011; Rajendran *et al*. 2011).

Bouremia *et al*. (2011) induced drought levels (0, -0.5, -1.0, -1.5, & -2.0 MPa) with PEG 6000 and reported the mean average root length of 22 sesame genotypes were 70.45mm for controls and 17.93 mm at an osmotic potential of -1 MPa after seven days of test period. He also observed that radicle lengths were longer under osmotic potential of -0.5MPa than under controlled conditions. According to Khan *et al*. (2019) the drought sensitive rapeseed cultivar ZY 36 showed lower root length compared with SG 127, the resistant cultivar under PEG treatment. Huang *et al*. (2017) reported a reduction in shoot and root length of *Eruca vesicaria* genotypes under 20 % PEG-6000 treatment.

According to Radhamani *et al*. (2012), PEG induced drought stress studies in castor recorded highest seedling root length in the genotype RG 3013 and RG 2326. Soyabean germplasm were evaluated using PEG for drought tolerance and it was observed that the genotypes Adonai, Neoplanta and PR92M22, followed by Celina recorded higher root length (Pavli *et al*., 2020).

As per the multivariate analysis conducted by Bibi *et al*. (2012) in sorghum, root length was the highest contributor (56.60 %) towards drought stress, so it can be utilized as a selection parameter at seedling stage in sorghum. The variation among genotypes for germination stress tolerance index (GSI) and root length stress index (RLSI) was found to be a reliable indicator to screen drought tolerant genotypes at germination and seedling stage in maize (Partheeban *et al*., 2017).

2.3.3.4 ***Shoot length***

Water stress induced by PEG 6000 had significantly reduced the radicle and coleoptile length of 2 sesame species studied, Safi Abadi and Dezfol (Bijeh, 2012). El-Harfi *et al*. (2016) reported that PEG induced drought stress negatively affected the shoot length in yellow and brown coloured sesame genotypes.

Shoot length decreased up to 80 % in soyabean genotypes subjected to drought at 15% PEG stress level (Kosturkova *et al*., 2008). Channaoui *et al*. (2019) reported that shoot and root length were decreased with increasing drought levels. PEG induced drought stress studies in castor showed that the mean seedling shoot length was maximum in the genotype RG2474 (16.40 cm) (Radhamani *et al*., 2012).

As per the multivariate analysis conducted by Bibi *et al*. (2012) in sorghum, shoot length contributes 20.20 % towards drought stress. Hellal *et al*. (2018) reported that the PEG treatment reduced shoot length of barley cultivars.

2.3.3.5 ***Speed of germination***

One of the important indexes for assessing drought stress tolerance is germination rate. Species with high germination rate are likely to grow rapidly than other spices under drought stress.

Bijeh, (2012) reported that in sesame species, germination percentage and speed of germination decline by osmotic potential enhancement. According to Kizil and Yol (2018) PEG induced water stress negatively affected the seedling parameters of sesame such as germination rate, shoot length and root length.

In barley cultivars germination speed was affected by drought stress more than germination percentage Hellal *et al*. (2018).

2.3.3.6 ***Vigour index***

According to Khodarahmpour (2011), germination percentage and seed vigour can be utilized as screening criteria for stress tolerance in plants. Vignesh *et al*. (2018) reported that PEG application at different concentrations (3%, 6%, 9%, 12%, 15%) decreased the vigour index of sesame varieties *viz*., CO 1, SVR1, SVPR 1, VRI-1, VRI-2, TMV3, TMV 4, TMV5, TMV6 and TMV7.

On the basis of germination study using PEG in sunflower, Ahmad *et al*. (2009) classified sunflower hybrids into three groups, G-101 and 64-A-93 the best

performing under drought conditions, Hysun33, Hysun-38 and SF-187 the hybrids having medium tolerance to water stress and S278 the sensitive one. Huang *et al*. (2017) reported that the shoot length, root length and vigour indices were higher in Eruca than in *B. napus* cultivar Zhongshuang 9 under PEG treatment.

Partheeban *et al*. (2017) reported that water stress induced by PEG under four levels of osmotic stress (0, -2, -4 and -6 bar) influenced the vigour index of different cultivars of maize *i.e*., VIM455, VIM147, VIM213 and VIM396. Drought stress induced using PEG-6000 (-0.1 bars to -2.0 bars) decreased the vigour index (*Cassia angustifolia*) at germination stage (7DAS).

2.4 MOLECULAR CHARACTERIZATION OF SESAME GENOTYPES

Despite its importance, sesame is considered as an orphan crop because it has received very little support from science, industry and policy makers. As a consequence, it lags behind the other major oilseed crops as genetic improvement is concerned (Dossa, 2016). The genetic and molecular biology study of sesame began very late. However, over the last few years, some significant progress has been made in the development of large-scale genomic resources including informative molecular markers, ultra-dense genetic maps, transcriptome assemblies, multi-omics online platforms *etc*. In addition, the release of the draft genome of sesame (Wang *et al*., 2014) triggered functional analyses of candidate genes related to key agronomic traits.

2.4.1 QTL associated with waterlogging tolerance in sesame

Sesame is highly susceptible to waterlogging stress. The crop experiences a reduction in growth and yield after 2–3 days of waterlogging (Ucan *et al*., 2007). Zhang *et al*. (2014) have mapped the QTL associated with waterlogging tolerance, using a recombinant inbred lines (RILs) population. They detected six QTLs (qEZ09ZCL13, qWH09CHL15, qEZ10ZCL07, qWH10ZCL09, qEZ10CHL07, and qWH10CHL09) related with waterlogging tolerance which were located on LG 7, 9, 13, and 15, explaining 5.67-17.19 per cent of the phenotype variation. Among the QTL, qWH10CHL09 on chromosome 9 was found to attribute maximum phenotypic variation (17.19 %) and the SSR marker ZM428 was closely linked with this QTL

(genetic distance was only 0.7 cM). He opined that the marker ZM428 can be used as effective marker for marker-assisted selection (MAS). ZM22, ZM92, E16M19, E14M14a, E5M12a, ZM351, M20E10, ZM428, E5M12a, ZM351and M20E10 were the other markers linked with the QLTs.

2.4.2 Genes associated with waterlogging tolerance

Wang *et al*. (2010a) isolated the genes related to waterlogging in sesame by suppression subtractive hybridization. The results showed that 84 sequences *i. e.* 79. 2 per cent of all the unigenes were homologous with the genes or proteins in GenBank. Thirty nine ESTs were related to basic material and energy metabolism, signal transduction, transcription regulation and detoxification defense response. Some of them were *Phosphoenolpyruvate carboxylase*, *Myo-inositol oxygenase*, *Galacturonic acid reductase* and *Xyloglucan endotransglycosylase*.

Wang *et al*. (2012) found 13,307 differentially expressed genes (DEGs) in sesame under waterlogging stress. In a more comprehensive study, a total of 1,379 genes were found as the core genes that function in response to waterlogging. They identified 66 genes that may be candidate for improving sesame tolerance to waterlogging (Wang *et al*., 2016).

2.4.2.1 Xyloglucan endotransglycosylase

*Xyloglucan endotransglucosylase/hydrolas*e has been recognized as a cell wall-modifying enzyme, participating in the diverse physiological roles. From water-stressed hot pepper plants, Cho *et al*. (2006) isolated three different cDNA clones (pCaXTH1, pCaXTH2, and pCaXTH3) that encode *Xyloglucan endotransglucosylase* homologs. RT-PCR analysis showed that three *Xyloglucan endotransglucosylase* mRNAs were concomitantly induced by a broad spectrum of abiotic stresses, including drought, high salinity and cold temperature, and in response to stress hormone ethylene, suggesting their role in the early events in the abiotic-related defense response. They also identified that over-expression of CaXTH3 improves drought and salt tolerance in Arabidopsis.

The involvement of *Xyloglucan endotransglycosylase* genes (NtEXGT) in the regulation of tobacco growth under hypothermia, drought, and salinity is also reported (Kulueve *et al*., 2019).

2.4.2.2. Myo-inositol oxygenase

Myo-inositol is a unique molecule that plays an essential role in the growth and development of eukaryotic cells. *Myo-inositol oxygenase* is an essential monooxygenase enzyme, catalyzes the transferring process of Myo-inositol into D-glucuronic acid, which is a vital sugar precursor for plant cell walls and plays a significant role in the production of hemicellulose precursors (Klinghammer and Tenhaken, 2007). *Myo-inositol oxygenase* maintains myo-inositol content necessary for the synthesis of some low molecular weight compounds in plants (Smirnoff *et al*., 2001). It is reported to be a critical enzyme in ascorbic acid biosynthetic pathway in plants (Lorence *et al*., 2004; Lisko *et al*., 2013). Studies indicate that it is crucial in abiotic stress tolerance and might be activated under sugar starvation conditions to generate alternative sugar sources, thereby indirectly contributing to metabolic homeostasis. Genome-wide analysis of *Myo-inositol oxygenase* gene family in tomato conducted by Munir *et al*. (2020) revealed their involvement in ascorbic acid accumulation.

2.4.2.3 Galacturonic acid reductase

D-Galacturonic acid reductase, a key enzyme in ascorbate biosynthesis. Hemavathi *et al*. (2010a) reported that over-expression of strawberry D-galacturonic acid reductase in potato leads to accumulation of vitamin C with enhanced abiotic stress tolerance. Enhanced tolerance was achieved in transgenic potato plants overexpressing *D-galacturonic acid reductase* gene in response to various abiotic stresses (Hemavathi *et al*., 2010b).

Agius *et al*. (2003) engineered increased vitamin C levels in plants by overexpression of a *D-galacturonic acid reductase* in strawberry plants.

2.4.2.4 Phosphoenol pyruvate carboxylase

In the leaves of C3 plants, *Phosphoenolpyruvate carboxylase* plays the anaplerotic role of replenishing tricarboxylic acid (TCA) cycle with intermediates that are consumed for a variety of biosynthetic pathways and nitrogen assimilation

(Izui *et al*., 2004; Leary *et al*., 2011). *Phosphoenolpyruvate carboxylase* has a wide range of non-photosynthetic roles including supporting carbon–nitrogen interactions, seed formation and germination, fruit ripening, guard cell metabolism during stomatal opening (Nakagawa *et al*., 2003), and provision of malate as a respiratory substrate for symbiotic N_2-fixing bacteroids in legume root nodules (Cousins *et al*., 2007).

Recently, accumulating evidence has confirmed that a large number of *Phosphoenol pyruvate carboxylase* genes are induced by abiotic and biotic stresses and play important roles in regulation of plant tolerance to stress (Cheng *et al*., 2016). Up-regulated *Phosphoenol pyruvate carboxylase* expression in response to salinity or drought stress has been well documented in C3, C4 and CAM plants (González *et al*., 2003). Overexpression of *Phosphoenol pyruvate carboxylase* gene in transgenic plants enhanced their tolerance to drought or salt stress, whereas knockdown lead to increased sensitivity to osmotic stress (Chen *et al*., 2010).

A comparative analysis between sesame of the tolerant and susceptible genotypes revealed 66 genes that may be candidates for improving sesame tolerance to waterlogging (Wang *et al*., 2016).

2.4.3 Tolerance to waterlogging and drought

The contrasting responses to drought and waterlogging suggest the existence of different underlying molecular mechanisms in sesame. Dossa *et al*. (2018) demonstrated that sesame has divergent epigenetic programs such as DNA methylation that respond to drought and waterlogging stresses results in the contrasting responds to drought and waterlogging.

Expression profiling in various tissues suggested that the *MYB* transcription factors (*SIMYBs*) are highly active in modulating sesame growth and development. Moreover, by integrating RNA-seq data and qRT-PCR analysis, Mmadi *et al*. (2017) demonstrated that *SIMYBs* are key transcription factors regulating sesame responses to drought and waterlogging stresses.

Li *et al*. (2017) conducted a comprehensive study on WRKY gene family in sesame and identified that 33 and 26 *SiWRKYs* respond strongly to waterlogging and

drought stress respectively. He also observed differential expression level of WRKY family genes in tolerant and sensitive genotypes.

2.4.4. Genes/QTLs offering waterlogging tolerance reported in other crops

A major QTL conferring waterlogging tolerance in Maize have been reported by Qiu *et al*. (2007) which is located near *sucrose synthase 1*, a known anaerobic response gene. The QTL designated as *Sub 1* controlling traits related to waterlogging tolerance have been mapped on many genomic regions (Xu and Mackill, 1996; Toojinda *et al.*, 2003) in rice. Xu *et al.* (2006) reported that the submergence tolerance gene, *Sub1A*, is an ethylene-response-factor-like gene that confers submergence tolerance to rice. Waterlogging studies in Soybean identified one QTL on chromosomes 6 and 15 and two QTL on chromosome 7 causing up to 30.7% phenotypic variation (Dhungana *et al*., 2021). Several genes related with fermentation pathways, hormones, aerenchyma formation, cell wall modifications, ROS balancing, calcium and nitric oxide signaling and photosynthetic adaptation were identified in different crops that impart waterlogging tolerance. *Sucrose synthese* gene in maize (Springer *et al*., 1986), *Alanine aminotransferase* in barley (Good and Crosby, 1989). *Glutamine synthase* in rice (Mattana *et al*., 1994), Calcium-dependent protein kinase (CDPK) in rice (Morello *et al*., 1994) are some of them.

2.5 WATERPROOFING OF SESAME WITH AMELIORANTS

2.5.1 Role of ameliorants in waterproofing

2.5.1.1 *Fertilizers for waterproofing (KNO_3, $Ca(NO_3)_2$, Urea)*

Nutrient deficiency is one of the major effects of waterlogging on plants, resulting in reduced photosynthesis and net carbon fixation, leading to a reduction in growth and yield (Bange *et al*., 2004). Waterlogging causes a significant decrease in nitrogen content and rate of nitrogen accumulation in plants due to reduced root activity. Yellowing of leaves due to loss of chlorophyll from leaves during waterlogging is attributed to nitrogen deficiency.

Application of essential nutrients will assist in mitigating the negative effects of abiotic stresses like waterlogging leading to increased productivity (Noreen *et al*.,

2018). A strategic use of nitrogen fertilizer prior to waterlogging may alleviate its deficiency (Rao *et al*., 2002). While the deleterious effects of waterlogging have often been partially counteracted by the addition of fertilizers mainly nitrogen, they did not fully overcome them due to the reduced ability of roots to absorb nutrients (Watson *et al.,* 1976). Hence foliar application of fertilizers plays an important role.

Potassium fertilizer has also been reported to ameliorate the detrimental effects of waterlogging in several crops including sugarcane (Sudama *et al.,* 1998), rapeseed (Cong *et al*., 2009) and cotton (Ashraf *et al.,* 2011). Pottassium supplement under waterlogging not only increased plant growth, photosynthetic pigments and photosynthetic capacity, but also improved plant nutrient uptake as a result of higher K^+ , Ca^{2+}, N, Mn^{2+} and Fe^{2+} accumulation (Ashraf *et al*., 2011). According to Pang *et al*. (2006), avoiding K^+ loss during hypoxia or anoxia stress is the key mechanism responsible for waterlogging resistance in plants.

Calcium (Ca) has a very important role, not only for cell wall and membrane stabilization, but is also involved in the regulation of specific plant responses to environmental stresses (Braam *et al*., 1996). Ca^{2+} inhibits or slows leaf tissue senescence through cross-linking pectates and cementing cell walls.

2.5.1.2. *Plant growth regulators for waterproofing*

Plant growth regulators are reported to mitigate waterlogging damage in plants by applying them at appropriate growth stage (Nguyen *et al*., 2018b; Ren *et al.,* 2018b; Wu *et al*., 2018).

2.5.1.2.1. *Salicylic acid*

Salicylic acid (SA) is a phenolic compound widely distributed in plants (Raskin *et al*., 1990). It acts as an important signaling molecule which mitigates the response of plants to environmental stress conditions (Shakirova *et al*., 2003). Increasing evidence showed that it plays important roles in diverse physiological processes in plants through induced level of cytokinin, gibberellin and also nitric oxide under waterlogging condition which can mediate stomatal movement (Garcia-Mata and Lamattina, 2002). Besides plant developmental processes, SA can also be involved in the regulation of plant defense mechanisms in responses to biotic and abiotic stresses.

2.5.1.2.2. *Tricyclazole*

Triazoles have both fungitoxic and plant-growth regulatory effects. They show protective role against various stresses. The triazoles have been characterized as plant multi-protectants (Leul and Zhou, 1998). Paclobutrazole, a triazole reported to mitigate waterlogging induced damage in canola and sweet potato plants (Lin *et al*., 2006).

2.5.1.2.3. *NAA*

Synthetic auxin, 1-naphthaleneacetic acid (1-NAA), was reported to promote the growth of adventitious roots in waterlogged barley plants (Pang *et al.,* 2007). It has been reported to improve plant growth under waterlogged conditions (Pang *et al*., 2007; Ren *et al*., 2016c). Increase in antioxidant ability and decrease in lipid peroxidation induced by the early ROS accumulation is reported to be triggered by NAA (Xing *et al*., 2016).

2.5.1.3. *Bio agents for waterproofing*

Pseudomonas fluorescens increases nutrient availability by solubilizing phosphatase and production of chelating substances like siderophores and also enhances activity of antioxidant enzymes enabling the plants to survive and maintain their growth under stress condition (Elekhtyar, 2015). They also facilitate the production of phytohormones such as indole acetic acid, gibberellic acid and cytokinins (Marschner and Timonen, 2006). The balanced synthesis of these hormones under stress improves plant growth. Saravanakumar *et al.* (2011) reported that *Pseudomonas fluorescens produce a* wide range of enzymes and metabolites that help plants to mitigate the harmful effects of various biotic and abiotic stresses.

2.5.2. Effect of ameliorants on waterproofing of morphological physiological and yield attributes

2.5.2.1. Morphological observations

2.5.2.1.1. *Plant height*

Ashraf *et al*. (2011) reported that under waterlogged conditions, foliar application of muriate of potash in cotton plants was more effective in enhancing stem length (37.1%) compared to normal conditions (31.1%). According to the experiment of Habibzadeh *et al.* (2013), foliar application of urea, calcium nitrate,

potassium nitrate and tricyclazole before waterlogging stress improved the shoot length in sesame compared to non-treated waterlogged plants. Jain *et al*. (2015) recorded an increase in plant height in waterlogged sugarcane at 5 leaf stage sprayed with urea, calcium nitrate or potassium nitrate. According to Singh *et al*. (2017) salicylic application in waterlogged tomato plants recorded 46 per cent increase in shoot length compared to untreated control.

2.5.2.1.2. *Root length*

Waterlogging stress had a marked inhibitory effect on root length of cotton plants. However, application of potassium either through rooting medium (soil), as foliar spray, or in combination significantly improved root length under normal as well as waterlogged conditions (Ashraf *et al*., 2011).

Habibzadeh *et al.* (2013) reported that foliar application of urea, calcium nitrate, potassium nitrate and tricyclazole before waterlogging stress increased the root length in sesame compared to non-treated waterlogged plants. The effect of urea on the increase in root length was less pronounced than that of the other compounds.

2.5.2.1.3. Root number

Pang *et al*. (2007) reported that foliar application of 1-NAA in 2 week old barley plants increased the production of adventitious roots.

2.5.2.1.4. Root and shoot dry weight

Habibzadeh *et al*. (2013) reported that foliar application of calcium nitrate, potassium nitrate and tricyclazole before and after waterlogging stress imposition increased the shoot and root dry weight in sesame compared to un-treated control. Jain *et al*. (2015) also reported an increase in shoot and root dry weight in waterlogged sugarcane of 5 leaf stage sprayed with urea, calcium nitrate, potassium nitrate.

Waterlogging stress significantly reduced stem and root dry weights in waterlogged cotton plants, while exogenous potassium (K) supplementation through soil, foliar and combined application showed 41.1, 45.2 and 126.9 per cent increase in shoot dry weight over control. Combined application also showed 74.3 per cent improvement in root dry weight over control (Ashraf *et al*., 2011).

2.5.2.1.5. Leaf number

Singh *et al*. (2017) recorded 19.56 per cent increase in number of leaves in waterlogged tomato plants sprayed with 50 ppm salicylic acid for 12 hr at 45 DAS.

2.5.2.2. Photosynthetic parameters

A reduction in photosynthetic rate, transpiration rate and stomatal conductance under waterlogged condition in cotton plants was reported by Ashraf *et al.* (2011). He observed that foliar application of K resulted in 105 per cent, 180 per cent and 15.6 per cent improvement in these parameters respectively. Pang *et al.* (2007) observed that application of 1-NAA in waterlogged barley cultivars allowed recovery of stomatal conductance to similar values to the control plants.

2.5.2.3. Enzyme activity in root

Foliar application of both potassium nitrate and calcium nitrate enhanced alcohol dehydrogenase gene expression in waterlogged sugarcane which indicates their significant role in waterproofing (Jain *et al*., 2015). According to Goyal *et al.* (2020) foliar application of KNO_3 reduced activities of pyruvate decarboxylase and alcohol dehydrogenase in roots of both tolerant and susceptible genotypes of maize.

2.5.2.4. Biochemical parameters

2.5.2.4.1. Catalase and nitrate reductase enzyme activity in leaves

Habibzadeh *et al.* (2013) reported that foliar application of urea, calcium nitrate, potassium nitrate and tricyclazole before waterlogging stress improved the catalase enzyme activity in sesame compared to non-treated waterlogged plants.

Jain *et al*. (2015) recorded an increase in nitrate reductase activity in waterlogged sugarcane sprayed with urea, calcium nitrate and potassium nitrate.

2.5.2.4.2. Total chlorophyll content

Total chlorophyll content was significantly improved (41.9%) under waterlogged conditions in cotton by exogenous K application (Ashraf *et al*., 2011). Ding *et al*. (2020) reported that foliar spray of urea in waterlogged wheat plants improved the chlorophyll content compared to the non-treated plants. It is reported that foliar spray of nitrogen ameliorated the effects of waterlogging by restoring chlorophyll concentration in the leaves of corn plants (Rao *et al*., 2002).

Application of urea to the waterlogged winter rape at seedling and stem elongation stage has been reported to improve the chlorophyll content (Zhou *et al*.,

1997). Singh *et al*. (2017) recorded 45 per cent increase in chlorophyll content in waterlogged tomato plants sprayed with salicylic acid. After imposing waterlogging stress, reduction in the chlorophyll content was observed in maize and salicylic acid increased its content (Yan *et al*., 1996).

2.5.2.4.3 *Soluble protein content*

El-Tayeb (2005) observed that increased protein content induced by salicylic acid might be helping in maintaining osmolarity in the cells during abiotic stress. Salicylic acid application increased the protein content in waterlogged tomato plants as reported by Singh *et al.* (2017).

2.5.2.4.4. *MDA content*

Habibzadeh *et al*. (2013) reported that foliar application of urea, calcium nitrate, potassium nitrate and tricyclazole before waterlogging stress decreases the MDA production in sesame leaves compared to non-treated waterlogged plants. A decrease in the production of MDA with foliar application of urea, calcium nitrate, and potassium nitrate in sugarcane has been reported by Jain *et al.* (2015).

2.5.2.5 Yield and yield attributes

Tomato plants sprayed with salicylic acid (SA) at 100 ppm increased yield and its components (Ali *et al.,* 2009). Similar results were obtained with SA in broad bean (Sanaa *et al*., 2001).Using 150 ppm of SA as a foliar application resulted the highest increment in number of pods and green pod yield of snap bean (Kmal *et al*., 2006). 36 per cent increase in fruit number was reported in waterlogged tomato plants spayed with SA (Singh *et al*., 2017).

Zhou *et al*. (1997) observed that application of nitrogen fertilizer in waterlogged winter rape improved yield components and seed yield.

Application of nitrogen, phosphorus and potassium fertilizers improved the number of seed per pod, siliques per plant, primary branches, secondary branches and the length of raceme in two rapeseed (*Brassica napus* L.) varieties (Zhongshuang No. 10 and Zhongyouza No. 5) subjected to waterlogging *at* seedling stage (Cong *et al.,* 2009). Reduction in grain yield owing to waterlogging could be effectively alleviated and even eliminated using exogenous nitrogen application in wheat under water logging (Ding *et al.,* 2020).

Materials & Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENT 1 (SURVEY)

A survey was conducted to identify constraints faced by sesame growing farmers in Kerala.

3.1.1 Location of survey

Alappuzha, Kollam and Thrissur districts were chosen for the study as the former two are the leading districts of sesame production in Kerala and the latter is the district with highest productivity. Ninety sesame growing farmers were selected from Kayamkulam (Alappuzha), Ochira (Kollam) and Koratty (Thrissur) for the survey.

3.1.2 Survey procedure

A questionnaire was prepared in consultation with farmers and experts dealing with sesame production and farmers were asked to rank these constraints based on the severity. The questionnaire prepared is given in Table 1.

3.1.3 Data analysis

The data collected were analyzed using Garrett's Ranking Technique. Prime advantage of this technique over simple frequency distribution is that the constraints are arranged based on their importance from the point of view of respondents. As per this method, respondents have been asked to assign the rank for all factors and the outcomes of such ranking have been converted into score value with the help of the following formula:

Garrett's formula for the conversion of ranks into percent:

Per cent position = $100 * (Rij - 0.5)/Nj$

Where, Rij = rank given for i^{th} constraint by j^{th} individual

Nj = number of constraints ranked by j^{th} individual

With the help of Garrett's Table (Table 2) given by **Garrett and Woodworth (1969)**, the per cent position estimated is converted into scores (Table 3). Then for each factor, the scores of each individual were added and then total value of scores and mean values of score is calculated. The factors having highest mean value is considered to be the most important factor.

Table 1. Questionnaire for farmer survey to identify constraints in sesame cultivation

FARMER SURVEY TO UNDERSTAND THE CONSTRAINTS FACED DURING SESAME PRODUCTION
Name :
Address:
Area :
Season:
Variety used:
Major crops cultivated?
Method of sowing?
1. Is there any problem of poor soil fertility?
2. Problem of unavailability of suitable variety
3. Poor seed germination?
4. Is there any problem of unavailability of quality seeds?
5. Weed infestation causing any problem in cultivation?
6. Whether disease and pest incidence caused trouble?
7. Have you ever faced any constraint due to excessive rain?
8. Is there any problem faced due to drought during cultivation?
9. Adequate labor is available?
10. High cost of labour?
11. Lack of adequate credit facilities?
12. Do you have any technical assistance from krishibhavan and research stations for the cultivation?
13. Problem related to harvesting, transport and drying including insufficiency of agricultural inputs or implements?
14. Problem during storage?
15. Any problem during marketing?
16. Is there any lack of good returns from sale?
17. Are you interested to continue sesame production?

Table 2. Garrett ranking conversion table

Percent	Score	Percent	Score	Percent	Score
0.09	99	22.32	65	83.31	31
0.20	98	23.88	64	84.56	30
0.32	97	25.48	63	85.75	29
0.45	96	27.15	62	86.89	28
0.61	95	28.86	61	87.96	27
0.78	94	30.61	60	88.97	26
0.97	93	32.42	59	89.94	25
1.18	92	34.25	58	90.83	24
1.42	91	36.15	57	91.67	23
1.68	90	38.06	56	92.45	22
1.96	89	40.01	55	93.19	21
2.28	88	41.97	54	93.86	20
2.69	87	43.97	53	94.49	19
3.01	86	45.97	52	95.08	18
3.43	85	47.98	51	95.62	17
3.89	84	50.00	50	96.11	16
4.38	83	52.02	49	96.57	15
4.92	82	54.03	48	96.99	14
5.51	81	56.03	47	97.37	13
6.14	80	58.03	46	97.72	12
6.81	79	59.99	45	98.04	11
7.55	78	61.94	44	98.32	10
8.33	77	63.85	43	98.58	9
9.17	76	65.75	42	98.82	8
10.06	75	67.48	41	99.03	7
11.03	74	69.39	40	99.22	6
12.04	73	71. 14	39	99.39	5
13.11	72	72.85	38	99.55	4
14.25	71	74.52	37	99.68	3
15.44	70	76.12	36	99.80	2
16.69	69	77.68	35	99.91	1
18.01	68	79.17	34	100.00	0
19.39	67	80.61	33		
20.93	66	81.99	32		

Table 3. Percent position and Garrett score of each rank

Rank	Percentile position	Garrett score
1	100 (1 -0.5)/ 9 = 5.55	81
2	100 (2 -0.5)/ 9 = 16.66	69
3	100 (3 -0.5)/ 9 = 27.77	62
4	100 (4 -0.5)/ 9 = 38.88	55
5	100 (5 -0.5)/ 9 = 50	50
6	100 (6 -0.5)/ 9 = 61.11	44
7	100 (7 -0.5)/ 9 = 72.22	38
8	100 (8 -0.5)/ 9 = 83.33	31
9	100 (9 -0.5)/ 9 = 94.44	19

3.2 EXPERIMENT 2

3.2.1 SCREENING OF SESAME VARIETIES FOR WATERLOGGING TOLERANCE

3.2.1.1 Location

A pot culture study for screening sesame genotypes for tolerance to water logging was conducted at College of Agriculture, Vellanikkara during summer (February-April) in 2018.

3.2.1.2 Varieties

The seeds of 15 sesame genotypes, collected from different research stations in India were used for the evaluation of waterlogging tolerance. The list of sesame genotypes used for the study is given in Table 4.

3.2.1.3. Experimental details

The fifteen Sesame varieties collected from different research stations were grown in pots. The pots were prepared by filling soil and vermicompost. Fertilizers were applied as per the Package of Practices Recommendations of Kerala Agricultural University (KAU, 2016). N: P: K @ 30:15:30 kg ha^{-1} as basal dose at the time of sowing. One week after sowing, plants were thinned and maintained 7 plants per pot.

Table 4. Sesame genotypes selected for evaluation

Sl. No.	Sesame genotypes	Releases from	Parentage	Duration (days)
1	Thilak	Kerala Agricultural University	Pureline selection from Malappuram local	80-99
2	Kayamkulam 1	Kerala Agricultural University	Pureline selection	90-100
3	Thilatara	Kerala Agricultural University	Combination breeding with parents B-9 x CST-785	78
4	Thilarani	Kerala Agricultural University	Thilak X Kayamkulam 1	80-99
5	Ayali	Local cultivar of Kerala	Local cultivar of Kerala	80-99
6	*Sesame malabaricum*	Wild sesame	Wild sesame	135
7	TMV-3	Tamilnadu Agricultural University	Derivative of South Arcot local X Malabar	80-85
8	TMV-4	Tamilnadu Agricultural University	Pureline selection from Sattur local	85-90
9	TMV-5	Tamilnadu Agricultural University	Pureline selection from Srivaikundam local	80-85
10	TMV-6	Tamilnadu Agricultural University	Pureline selection from Andhra Pradesh Variety	85-90
11	TMV-7	Tamilnadu Agricultural University	Derivative of SI 250 X ES 22	80-85
12	SVPR-1	Tamilnadu Agricultural University	Pureline selection from Western Ghat	75-80
13	CO-1	Tamilnadu Agricultural University	Derivative of (TMV 3 X SI 1878) X SI 1878	85-90
14	AT-231	Junagath Agricultural University, Gujarat	Hybridization of AT 90 X AT 104 followed by pedigree breeding	91
15	GT-10	Junagath Agricultural University, Gujarat	Selection from TNAU17	105

3.2.1.4 Treatments

The experiments includes two sets

1. Waterlogging at vegetative stage (20 DAS) with non-waterlogged control plants
2. Waterlogging at flowering stage (40 DAS) with non-waterlogged control plants

In one set of pots waterlogging was imposed at vegetative stage (20 DAS) and in another set at flowering stage (40 DAS) of 15 sesame genotypes. Period of waterlogging was 72 hr. During these 72 hrs the water was maintained at 2 cm from the soil surface. At the end of 72 hr, water was drained from pots.

3.2.1.5 Design of experiment

Completely Randomized Design with four replications

3.2.1.6 Observations recorded

3.2.1.6.1. *Survival percentage (%)*

Two weeks after the end of waterlogging treatment the number of seedlings survived was counted and survival per cent was calculated as:

$$\text{Survival percentage} = \frac{\text{Number of seedlings survived}}{\text{Total number of seedlings}} \times 100$$

3.2.1.6.2. *Plant height (cm), Root length (cm) and Root number per plant*

One week after imposition of waterlogging, sample plants were pulled out carefully (one week after waterlogging) without damaging the root system and length of primary root was measured as root length and expressed in centimetres. Tap roots, laterals and adventitious roots produced from each sample plant was measured and expressed as root number per plant. Height of above ground part was measured as plant height at one month after waterlogging.

Plate 1. Screening of sesame genotypes for waterlogging tolerance

3.2.1.6.3. Root and shoot dry weight (g)

Samples were oven dried and dry weight was taken using weighing balance for shoot and root separately.

3.2.1.6.4. Photosynthetic rate (µ mol $m^{-2}s^{-1}$), Transpiration rate (m mol $m^{-2}s^{-2}$) and Stomatal conductance (m mol $m^{-2}s^{-1}$)

Photosynthetic rate, transpiration rate and stomatal conductance were measured using infrared gas analyzer (Model LI-6400 of Licor Inc. Lincoln, Nebraska, USA) at the end of waterlogging period both at 20DAS and 40DAS. Readings were taken from 8 to 10 am.

3.2.1.6.5. Lactate dehydrogenase (LDH) (unit g^{-1} protein), Alcohol dehydrogenase (ADH) (unit g^{-1} protein) and Pyruvate decarboxylase (PDC) (unit g^{-1} protein)

Root sections (weighing ~0.5 g) were harvested at the end of each waterlogging treatment, snap frozen in liquid nitrogen, ground to a powder and stored at −70 ◦C until use. The material was extracted in 50 mM Tris–HCl, pH 6.8, containing 5 mM $MgCl_2$, 5 mM mercaptoethanol, 15 per cent (v/v) glycerol, 1 mM EDTA, 1 mM EGTA and 0.1 mM pefabloc proteinase inhibitor. LDH activity was measured by spectrophotometric monitoring of NADH oxidation during the conversion of pyruvate to lactate at 340 nm in a reaction mixture composed of 0.1 M phosphate buffer, pH 7.0, 4 M NADH, 0.24 mM pyruvate, the reaction was initiated with addition of the extract (Vassault, 1983). ADH and PDC were also measured spectrophotometrically by monitoring the oxidation of NADH at 340 nm as described by Waters *et al*. (1991).

3.2.1.6.6 Catalase enzyme activity (µ mol of H_2O_2 utilized $g^{-1}min^{-1}$)

Catalase activity was measured by the method of Maehly (1954). One gram of leaf sample was homogenized in 10 ml of 0.1 M sodium phosphate buffer pH 7 and centrifuged at 4^0C for 10 min at 10,000 rpm. An aliquot of 1 ml of the supernatant of the enzyme extract was added to the reaction mixture containing 1 ml of 0.01 M

H_2O_2 and 3 ml of 0.1 M sodium phosphate buffer. The reaction was stopped after an incubation of 5 min at 20^0C by adding 10 ml of 1% H_2SO_4. The acidified medium without or with the enzyme extract was titrated against 0.005 N $KMnO_4$ and catalase activity was expressed as µ mol of H_2O_2 utilized g^{-1} fr. wt. min^{-1}.

3.2.1.6.7. Nitrate reductase enzyme activity (µg NO^-_2 g^{-1} h^{-1})

Nitrate reductase activity from plant leaf was measured by the method of Hageman and Flesher (1960). The produced nitrite was estimated by adopting the method of Nicholas *et al*. (1976), by measuring the absorbance of pink colour at 540 nm using spectrophotometer and expressed in µ moles of NO_2^- formed g^{-1} fresh weight hr^{-1}.

3.2.1.6.8. Chlorophyll content (mg g^{-1})

The total chlorophyll content in leaf was estimated using the DMSO method suggested by Hiscox and Israelstam (1979). The absorbance was measured in spectrophotometer (Model-4001/4 Thermo Spectonic, Thermo Electron Corporation, USA) at two wavelengths *i.e*., 663 nm and 645 nm and expressed as mg g^{-1} fresh weight of leaf tissue. The calculation was done as follows.

Total chlorophyll content = [(20.2 x A645) + (8.02 x A663)] x V/1000 x W

Where,

A = Absorption at given wavelength
V = Total volume of sample in extraction medium
W = Weight of leaf sample

3.2.1.6.9. Soluble protein content (mg g^{-1})

Total soluble protein content in the leaf was estimated by the method suggested by Lowry *et al.* (1951). 500 mg of leaf sample was macerated with 10ml phosphate buffer solution. After centrifugation for 10 minutes at 3000rpm the volume of supernatant was made up to 25ml. 5ml of ACT (alkaline copper tartarate) and 0.5ml of Folin-Ciocalteau reagent were added to 1ml of supernatant. After 30

minutes absorbance of the blue colour at 660nm was measured in a spectrophotometer and expressed as mg g^{-1}.

3.2.1.6.10. MDA (malondialdehyde) content (nmol g^{-1})

MDA content was measured following the method of De Vos *et al*. (1991). Powdered samples were homogenized in 0.1 per cent trichloroacetic acid. Supernatent collected after centrifugation was added with 0.5 per cent thiobarbituric acid. Then heated at 90^0C for 30 min. The amount of red coloured MDA formed was measured spectrophotometrically at 532 nm.

3.2.1.6.11 Anatomical study

Mature adventitious roots of the selected plants were collected and washed thoroughly in tap water for anatomical studies. Very thin cross sections were taken using fresh stainless steel blade and kept in a watch glass for staining with safranin (2%). The cross sections were kept in the diluted stain for two minutes followed by washing in distilled water. These sections were observed under the Motic microscope. Photographs of cross sections in the desirable fields were captured and analyzed.

3.2.1.7 Statistical analysis

Statistical analysis of the data was carried out using WASP 2.0, developed by ICAR, GOA. Pair wise comparisons of the treatments were done using critical difference. The control and treatment means were compared using t test.

3.2.2.. EXPERIMENT 3 (SCREENING FOR TOLERANCE TO DROUGHT STRESS)

A laboratory study was conducted to screen sesame genotypes for tolerance to drought at seedling stage. The details of the study are given below.

3.2.2.1 Location

The lab study was undertaken in the Department of Plant Physiology, College of Agriculture, Vellanikkara during June 2020.

3.2.2.2 Varieties

All the fifteen sesame genotypes (Table 4) selected for the evaluation of waterlogging tolerance were also screened for water stress tolerance.

3.2.2.3 Experimental Design

Completely Randomized Design with 4 replications

3.2.2.4 Screening procedure and treatments

Germination test was carried out as per the procedure outlined in ISTA rules (1999) to screen sesame genotypes for tolerance to drought at seedling stage. Four replications of 100 seeds of each genotypes were sown in petri plates with filter paper as a medium for germination.

Screening for drought tolerance was done using aqueous solution of PEG 6000 by the gradual stress induction method of Bangi *et al*. (2020) under laboratory condition. The seeds were sown in petriplates and were subjected to 0 MPa PEG (water) for 24 hr, followed by -0.5 MPa PEG for 24 hr, followed by -1MPa PEG for 24 hr, followed by -1.5 MPa PEG for 24 hr followed by -2MPa PEG for 24 hr followed by 0Mpa for 48 hr. A control set was maintained which is supplied with distilled water instead of PEG. At the end of test period of seven days the germination parameters were recorded. -0.5MPa PEG,-1MPaPEG, -1.5MPa PEG and 2MPa PEG was prepared by dissolving 4.24g, 4.75g, 5.26g and 5.77g PEG respectively in 50ml of distilled water.

3.2.2.5 Observations recorded

3.2.2.5.1 ***Normal seedlings (%)***

Normal seedlings are seedlings which possess the essential structures that are indicative of their ability to produce healthy plants under favorable field conditions. After the test period of seven days, the normal seedlings were counted and the mean values were expressed as percentage of the total number of seeds placed for germination.

3.2.2.5.2 Abnormal seedlings (%)

At the end of test period of seven days the number of abnormal seedlings such as damaged, decayed and deformed seedlings were counted and the mean values were expressed as percentage of total number of seeds placed for germination.

3.2.2.5.3 Hard seeds (%)

Seeds which do not absorb moisture till the end of the test period and remain hard were counted and mean values were expressed as percentage of total number of seeds placed for germination.

3.2.2.5.4 Dead seeds (%)

At the end of test period, seeds that are neither hard or nor fresh or have not produced any part of a seedling were counted and the mean values were expressed as percentage of total number of seeds placed for germination.

3.2.2.5.5. Fresh and ungerminated seeds (%)

Seeds which are neither hard nor have germinated but remain firm and apparently viable at the end of the test period were counted and the mean values were expressed as percentage of total number of seeds placed for germination.

3.2.2.5.6 Shoot length (cm)

Shoot length of germinated seedlings was measured and mean value was expressed in centimetres.

3.2.2.5.7 Root length (cm)

The root lengths of seedlings were measured as the length between the collar and the tip of the primary root and the mean length was expressed in centimetres.

3.2.2.5.8 Speed of germination

Speed of germination was calculated using the following formula given by Maguire (1962). The results were expressed in number.

Speed of germination = X1/Y1 + X2-X1/Y2+…..+ Xn – Xn-1/ Yn

X1- Number of seeds germinated at first count

X2- Number of seeds germinated at second count

Xn- Number of seeds germinated at n th day

Y1- Number of days from sowing to first count

Y2- Number of days from sowing to second count

Yn- Number of days from sowing to nth count

3.2.2.5.9 Vigour index

Vigour index-I was computed in the present experiment by using the procedure of Abdul-Baki and Anderson (1973).

Vigour index-I = Germination (%) × Total seedling length (cm)

3.2.2.5.10 Stress tolerance index

Stress tolerance index was calculated using formula given by Dhopte and Livera (1989).

Stress tolerance index = (Vigour index of treated seedling/ vigour index of control seedlings) x 100

3.2.2.6 Statistical analysis

Statistical analysis of the data was done using WASP 2.0, developed by ICAR, GOA. Pair wise comparisons of the treatments were done using critical difference.

3.2.2.7 Cluster analysis

The hierarchical cluster analysis of 15 genotypes with characters related to waterlogging were carried out. The genotypes were grouped into four clusters based on 'Euclidean distance' and the linkage method used was 'Average linkage method' and the cluster dendrogram was constructed using R software.

3.3. EXPERIMENT 3

CHARACTERIZATION OF GENOTYPES USING REPORTED MARKERS FOR SUBMERGENCE TOLERANCE IN SESAME

For conducting the molecular characterization of sesame genotypes to waterlogging, the genomic DNA was isolated and the candidate genes were PCR amplified using the specific primers (4 designed and 2 reported primers). The PCR products were sequenced, aligned in MAAFT and characterized.

3.3.1 Sesame genotypes

The sesame genotype *S. malabaricum* which was found to be tolerant to both stress (waterlogging, drought), the genotypes Ayali, Thilarani, Kayamkulam1 found to be moderately tolerant to both stress, CO 1 which is susceptible to both stress, Thilatara which is susceptible to waterlogging but tolerant to drought and TMV 5 which was tolerant to drought but susceptible to waterlogging were selected for the study.

3.3.2 Genes/markers used

The two SSR markers ZM428 (closely linked) and ZM22 linked to QTL for waterlogging tolerance (Zhang *et al*., 2014) were used for evaluating the genotypes for submergence tolerance. Additionally, the genes *Phosphoenol pyruvate carboxylase, Inositol oxigenase, Xyloglucan endotransglycosylase* and *Galacturonate reductase* which are reported to be involved in waterlogging tolerance in sesame (Wang *et al*., 2010a) were also characterized.

3.3.3 Genomic DNA isolation

The genomic DNA from leaf tissue was isolated by CTAB method (Rogers and Bendich, 1985).

3.3.3.1 Reagents

1. Liquid nitrogen
2. Polyvinylpyrrolidone (PVP)
3. 5x CTAB extraction buffer
   - 5 per cent CTAB (w/v)
   - 100 mM Tris (pH 8.0)
   - 20 mM EDTA (pH 8.0)
   - 1.4 M NaCl
   - 1 per cent PVP
4. TE buffer
   - 10 mM Tris (pH 8.0)
   - 1 mM EDTA
5. β-mercaptoethanol
6. Chloroform: isoamyl alcohol (24:1)

7. Isopropanol (chilled)
8. Ethanol (100 and 70 %)
9. Distilled water
10. RNase (1%)

3.3.3.2 DNA isolation

Fresh and clean leaf tissue (200 mg) was ground in a pre-chilled mortar and pestle with liquid nitrogen and a pinch of PVP. It was then suspended in CTAB buffer in an autoclaved centrifuge tube and 50 µl β-mercaptoethanol was added. Then it was mixed properly and incubated at 65 °C for 30 min. in a water bath with occasional mixing by gentle inversion followed by 10 min. incubation on ice. Chloroform:isoamyl alcohol (24:1) in equal volume was added by gentle mixing and inverting the tube. The tubes were centrifuged at 12,000 rpm for 15 min. at 4 °C and the extract was separated in to three distinct phases. The genomic DNA from top aqueous phase was transferred into another microcentrifuge tube. RNase treatment was given by adding 1 µl RNase at 37 °C for 45 min. After that, an equal volume of chloroform: isoamyl alcohol (24:1) was added, vortexed for 5 sec. and centrifuged at 12,000 rpm for 15 min. at 4 °C. Top aqueous phase was transferred to a new microcentrifuge tube. Chilled isopropanol 0.6 volume was added and mixed by quick gentle inversion till the DNA was precipitated. Incubated at -20 °C for 2 h or overnight and then the tube was centrifuged at 10,000 rpm for 10 min. at 4 °C. The supernatant was discarded and the pellet containing DNA was washed first in 70 % ethanol and then by 100 % ethanol, spun for 3 min. at 8,000 rpm, and discarded the ethanol. Air dried the pellet long enough to remove alcohol, but without completely drying the DNA and was dissolved in 50 µl of TE buffer and stored at -20 °C.

3.3.4. Assessing the quality of DNA by electrophoresis

3.3.4.1 Reagents used

1. Agarose (1%)
2. 50 X TAE buffer (pH 8.0)
   Tris buffer (1 M)
   Glacial acetic acid
   0.5 M EDTA

3. Tracking/ loading dye (6X)
4. Ethidium bromide

3.3.4.2 Electrophoresis

1. The gel casting tray was prepared by sealing the ends with tape. Comb was placed in gel tray about an inch from one end of the tray and positioned the comb vertically such that the teeth was about 1-2 mm above the surface of the tray.
2. The gel was prepared by adding 1 g of agarose in 100 ml of 1X TAE buffer in a glass conical flask. The mixture was heated in a microwave oven until all the agarose particles were completely dissolved and a clear solution was obtained.
3. The solution was allowed to cool down to 42-45 °C and an appropriate amount of ethidium bromide was added and mixed well. The warm gel was then poured into gel casting tray and left to solidify for 20 min. at room temperature.
4. Special care was taken to avoid any air bubbles near the wells or on the gel.
5. Once the gel was solidified, a small amount of 1 X TAE was poured on top of the gel and the comb was removed carefully without breaking the gel. The TAE solution was discarded and the gel along with the tray was kept inside the electrophoresis tank with the wells on the negative electrode side.
6. The electrophoresis tank was filled with 1 X TAE sufficient enough to submerge the wells.
7. The samples to be electrophoresed were prepared by mixing 5 µl of the DNA sample with 1 µl of 6X gel loading dye. After mixing, the total volume of 6 µl was loaded into individual wells.
8. The samples were electrophoresed at 75V until gel tracking dye reached 2/3rd of the gel length.

3.3.5 Gel documentation

Documentation of the electrophoresed gel was done under UV with a gel documentation system (Gel Doc, Uvitech, Cambridge) using Fire Reader software.

3.3.6 Assessing the quality and quantity of DNA by NanoDrop method

NanoDrop^{R} ND-1000 spectrophotometer was used for the estimation of quality and quantity of genomic DNA. The instrument was set to zero as blank using 1 µl autoclaved distilled water. Then 1 µl of DNA sample was loaded onto the

pedestal. The absorbance observed at 260nm and 280nm wavelength and the ratio of A_{260}/A_{280} were recorded to assess the purity of DNA.

Quality of DNA obtained is categorized as good if the ratio of A_{260}/A_{280} is between 1.8 and 2.0. The quantity of DNA present in the sample was calculated using the formula,

A_{260} = 1 is equivalent to 50 µg double-stranded DNA/µl sample

3.3.7 Primer designing

Nucleotide sequences of the selected genes were retrieved from NCBI genbank. ORFs of the sequence were identified using ORFfinder. Specific portion of the nucleotide that contain maximum ORFs were selected. Primer was designed for this region using Primer3. Primers of the reported SSR markers were obtained from the literature (Zhang *et al*., 2014). The list of primers are given in Table 5.

Table 5. Primers used for the study

Sl. No.	**Genes/markers**	**Primer sequence (Forward and Reverse)**	**No. of bases**	**Reference**
1	*Phosphoenol pyruvate carboxylase*	5'CTCCCTTCTGACTGCTTTGG 3' 5' TTCCTCATAGTTGGCCCTCA 3'	20	Wang *et al*. (2010)
2	*Inositol oxigenase*	5' GAATGCAATGCAGGTGAAGA 3' 5 AAAGGGCACTTTTCAGACCA 3'	20	
3	*Xyloglucan endotransglycosylase*	5' GCTCTGGTTTCCAGTCCAAG 3' 5' ATAGTCCCCACGAACACACC 3'	20	
4	*Galacturonate reductase*	5' AGCCATGCAACTCAAAATCG 3' 5' TCATTTGAACGGCTACCACA 3'	20	
5	SSR-ZM428	5' AGGATGATGATGTGATGAGAG 3' 5' CTGCTACTCCTT TTGTCTCTG 3'	21	Zhang *et al*. (2014)
6	SSR-ZM22	5' ACCACCGATCTACTCACTTTT 3' 5' CCACTGCACACTACAGTTTTT 3'	21	

3.3.8 Polymerase chain reaction

Polymerase chain reaction was carried out for DNA amplification by using SimpliAmp thermal cycler. A reaction mixture was prepared as shown below

3.3.8.1 Composition of reaction mixture for PCR

Materials	**Quantity**
Genomic DNA -	1µl
Takara Master Mix-	25 µl
Primer -	5 µl each
Sterile distilled water -	14 µl
Total volume	**50 µl**

The thermo cycler was programmed as follows:

For designed primers

- Initial denaturation- 94 °C for 5 min.
- Denaturation- 94 °C for 30 sec.
- Primer annealing- 60 °C for 30 sec.
- Primer extension- 72 °C for 1 min 30 sec.

} 35 cycles

- Final extension at 72 °C for 10 min.
- Incubate at 4 °C

For reported primers

- Initial denaturation - 94 °C for 5 min.
- Denaturation - 94 °C for 30 sec.
- Primer annealing - 54 °C for 30 sec.
- Primer extension - 72 °C for 50 sec.

} 35 cycles

- Final extension at 72 °C for 5 min.
- Incubate at 4 °C

3.3.9 Elution of DNA from the gel

GFX^{TM} PCR DNA and Gel Band Purification Kit were used for elution as per the manufacturer's instructions. DNA band to be eluted was excised out from agarose gel with a scalpel blade into a pre-weighed autoclaved microcentrifuge tube. Weight of gel band was calculated and capture buffer was added in the ratio of 10 µl

of buffer for each 10 mg of gel slice. The sample was mixed vigorously by vortexing and incubated at 60°C until agarose dissolved completely (approximately 15 minutes). The tube was then centrifuged briefly and supernatant was transferred to a GFX column placed inside a collection tube. This was incubated at room temperature for 1 minute and then centrifuged at maximum speed in a microcentrifuge for 30 sec. The flow-through was discarded from the collection tube and 500 µl of Wash buffer was added to GFX column. This was centrifuged for 1 minute at maximum speed in a microcentrifuge. This column was then transferred to a fresh microcentrifuge tube and 50 µl of sterile double distilled water was added onto glass fibre matrix. The sample was incubated at room temperature for 3 minutes and centrifuged at maximum speed in a microcentrifuge for 1 minute. This step caused elution of DNA sample into the fresh tube. Concentration of the purified DNA sample was checked by agarose gel electrophoresis.

3.3.10 DNA Sequencing

Purified DNA was Sanger sequenced at Eurofins Genomics India Pvt. Ltd., Bangalore. Total of 28 samples from seven genotypes were sequenced.

3.3.11 Sequence similarity search

The forward and reverse sequences of all the loci were aligned to generate the contigs and the contigs were analysed using BLASTn to find the similarity with the GenBank sequences.

3.3.11 Sequence alignment

Nucleotide sequences of each gene obtained from tolerant and susceptible genotypes were aligned separately using MAAFT and compared for any difference among the genotypes for the particular gene.

3.3.12 SNP analysis

Sequences of each gene from tolerant and susceptible cultivars aligned were analysed carefully to identify the single nucleotide polymorphisms (SNPs). SNPs conserved among the tolerant and susceptible cultivars were considered as the candidate SNPs for the trait expression. The effect of these candidate SNPs in protein

translation was examined and for this, translation from each frame were analysed using Expasy translate tool. The conserved SNPs leading to termination codon were considered as the potential SNPs contributing to the trait.

3.4. EXPERIMENT 4

AMELIORATION OF WATER LOGGING STRESS IN SESAME

(POT CULTURE EXPERIMENT)

A pot culture experiment was carried out to evaluate ameliorative role of some foliar and seed treatments on mitigating the negative effect of waterlogging on sesame growth.

3.4.1 Location

A pot culture study was conducted at College of Agriculture, Vellanikkara during February to April 2019.

3.4.2 Variety

Sesame variety Thilak, released from Kerala Agricultural University was selected for the study.

3.4.3 Experimental design

Experiment was laid out in Completely Randomized Design with four replicates.

3.4.4 Experimental details

Pots were prepared by filling soil and vermicompost. Fertilizers were applied as per Package of Practices Recommendations of Kerala Agricultural University (KAU, 2016). N: P: K @ 30:15:30 kg ha^{-1} as basal dose at the time of sowing. Waterlogging was imposed at 20 DAS and maintained 2 cm level from soil surface for 72 hrs. Following are the treatments tried.

3.4.5 Treatments (T) :

T_1- 1- Naphthalene acetic acid (NAA) (5 µg in 200µL)

T_2- Urea (1500 mg L^{-1})

T_3- Calcium nitrate (4100 mg L^{-1})

T_4- Potassium nitrate (5000 mg L^{-1})

T_5- Tricyclazole (50 mg L^{-1})

T_6- Salicylic acid (100 ppm)

T_7- *Pseudomonas fluorescens*

T_8- Control 1-Waterlogged control without ameliorants

T_9 – Control 2- No waterlogging and no ameliorant

Treatments, T_1, T_2, T_3, T_4, T_5, and T_6 were applied as foliar spray at 2 days before water logging. For treatment T_7, seed priming of talc formulation (10g L^{-1}) of *Pseudomonas fluorescens* followed by foliar spray of culture broth (30 mL L^{-1}) at 2 days before water logging was adopted.

3.4.6 Observations recorded

Morpho-physiological and biochemical observations as in experiment 2 were recorded.

3.4.7 Statistical analysis

Statistical analysis of the data was done using WASP 2.0, developed by ICAR, GOA. Pair wise comparisons of the treatments were done using critical difference.

Plate 2. Pot culture study for selection of best ameliorants under water logged condition in sesame var. Thilak

3.5 EXPERIMENT 5

FIELD EVALUATION OF BEST 3 AMELIORANTS

A field study was conducted to evaluate best three ameliorative treatments selected based on survival per cent in experiment 5. The materials and methodology adopted in the field study are briefly described below.

3.5.1 Location

Field study was conducted at farmer's field in Cherthala at Alappuzha district during February to May 2019.

3.5.2 Variety used

Sesame variety used for the field study was Thilak (ACV3) released from Kerala Agricultural University (1993). Thilak is a pureline selection from Malappuram local.

3.5.3 Design of experiment

The field was laid out in Randomized block design with five treatments and four replications.

3.5.4 Land preparation and sowing

Experimental field was prepared for conducting waterlogging study by placing polythene sheet 35 cm below the soil surface so that each plot can be waterlogged. The experimental area was ploughed to a fine tilth and levelled. The field consisted of 20 plots with a size of $2m^2$ (2m×1m) and a bund size of 40 cm between plots. Farmyard manure and fertilizers were applied as per the Package of Practices Recommendations of Kerala Agricultural University (KAU, 2016). N: P: K @ 30:15:30 Kg ha^{-1} was applied as basal dose at the time of sowing. Seeds were dibbled at a spacing of 15 cm ×20 cm. Thinning was done 15 days after sowing. Waterlogging was imposed in the field at 20 DAS for 72 hrs. The ameliorative treatments were applied 2 days before waterlogging.

3.5. 5 Treatments

T_1: Potassium nitrate (KNO_3) (5000 mg L^{-1})

T_2: *Pseudomonas fluorescens*

T_3: Salicylic acid (100 ppm)

T_4: Control 1- Waterlogged control without ameliorants

T_5: Control 2- No waterlogging and no ameliorants

Potassium nitrate and Salicylic acid were applied as foliar spray at 2 days before imposition of waterlogging. *P. fluorescens* was applid as seed priming of talc formulation (10g L^{-1}) at sowing followed by foliar spray of culture broth (30 mL L^{-1}) at 2 days before water logging.

3.5.6.1 Morphological observations

3.5.6.1.1 Survival percentage

Two weeks after the end of waterlogging treatment the number of plants survived was counted and per cent was calculated by dividing the total plant population of each treatment plot.

3.5.6.1.2 Days to 50 per cent flowering

Number of days for 50 per cent flowering in each treatment plot were observed and recorded.

3.5.6.1.3 Plant height (cm)

Four plants were chosen randomly from each experimental plot and tagged. Height of selected plants were measured at harvest. Measurement was taken from the base to the tip of the stem and expressed in centimeters.

3.5.6.1.4 Leaf number

Number of leaves in the tagged plants in each treatment plots were counted.

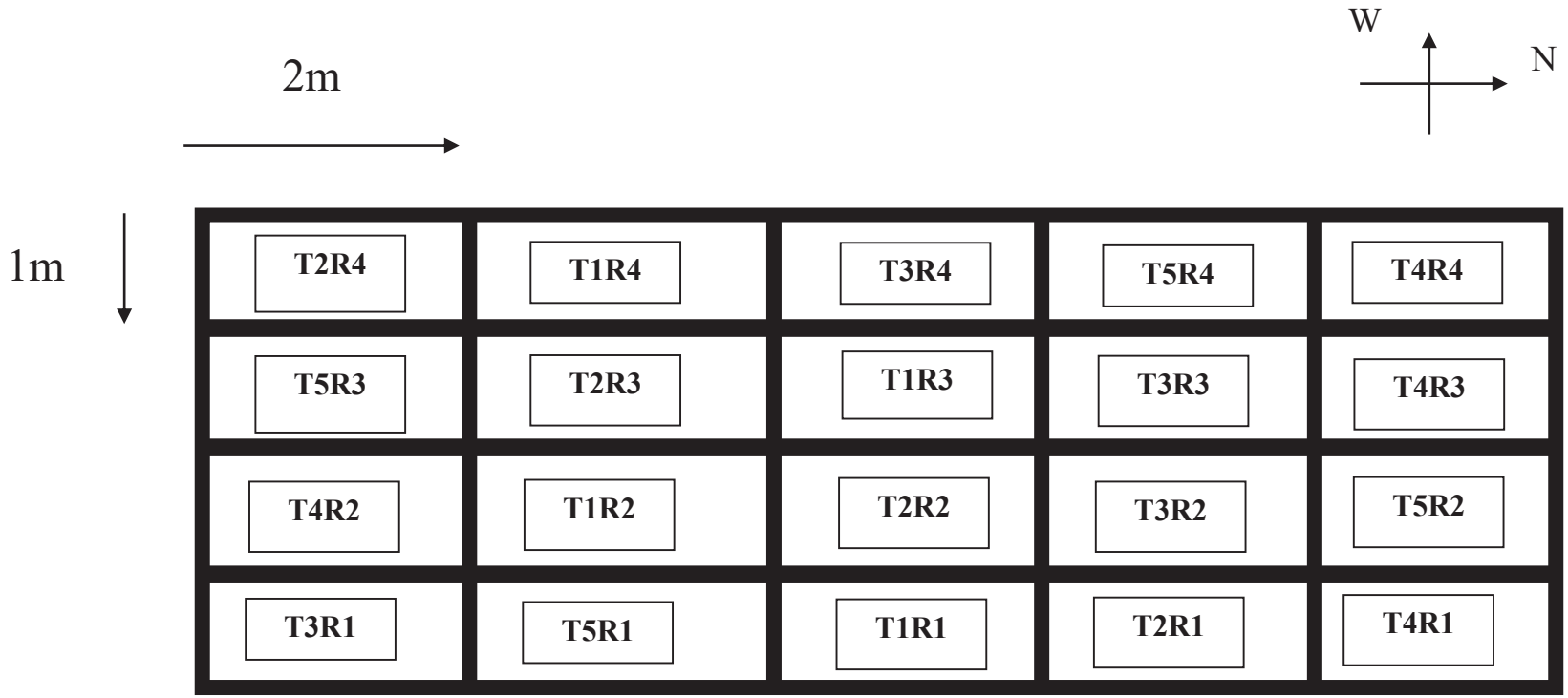


Fig 1. Layout of field experiment

T1 : Potassium nitrate (5000 mg L^{-1})

T2 : *Pseudomonas fluorescens*

T3 : Salicylic acid (100 ppm)

T4 : Waterlogged control without ameliorants

T5 : No waterlogging and no ameliorants

3.5.6.1.5. Root and shoot dry weight (g)

At the time of harvest plant samples were taken and oven dried. The dry weight was taken using electronic weighing balance for shoot and root separately.

3.5.6.2 Yield and yield attributes

3.5.6.2.1 No. of branches per plant

The number of branches per plant of selected plants in each plot was counted at the time of harvest.

3.5.6.2.2 No. of capsules per plant

The number of capsules of selected plants in each plot was counted at the time of harvest.

3.5.6.2.3. No. of seeds per capsule

At the time of harvest, number of seeds in each capsule was counted from ten pods of each plot and the mean was estimated for each variety.

3.5.6.2.4 1000 seed weight

One thousand seeds were counted from pods of selected plants in each replication and their weight was taken and recorded in grams.

3.5.6.2.5 Yield per plant (g)

The harvested seeds from plants of each plot were weighed separately and the mean value was expressed as grams per plant.

3.5.6.2.6 Yield per hectare (kg ha^{-1})

The yield obtained from each plot was estimated and expressed as kg per hectare.

Plate 3. Land preparation for field experiment

Plate 4. Sowing of sesame seeds for field study

Plate 5. Waterlogging of experimental field

Plate 6. General view of experimental field during waterlogging

3.5.7 Statistical analysis

Statistical analysis of the data was conducted using WASP 2.0, developed by ICAR, GOA. Pair wise comparisons of the treatments were done using critical difference.

3.6. Data analysis for identification of superior genotypes tolerant to waterlogging and drought stress

The growth parameters estimated from waterlogging and drought stress experiments were subjected for Z-distribution analysis using the formula,

Z value = (Specific mean-General mean)/ Standard deviation

The values calculated using data from the two stresses were plotted as X-Y scatter to distribute the data points in four quadrants (Ramu *et al*., 2012).

Results

4. RESULTS

4.1 FARMER SURVEY

Sesame (*Sesamum indicum* L.) is a valued oil crop of India and also an important agricultural export commodity. However, sesame provides only a small contribution to the total area of oil seeds in Kerala. As the sesame cultivation of the state has been declining, identification of constraints faced by farmers is an urgent need. Hence 30 farmers in three districts *viz*., Alappuzha, Kollam and Thrissur were surveyed and the constraints were ranked using Garrett ranking technique. The details of constraints are given below

4.1.1 Constraints faced by sesame farmers in Kerala

High labour cost, excessive rain fall, drought, weed infestation, unavailability of labour, pest and disease, marketing problem, problems for transportation, drying and threshing, and storage problems were the identified constraints of sesame growing farmers in Kerala. The garret score and ranking of the constraints are given in Table 6.

Table 6. Constraints faced by sesame farmers in Kerala

Sl. No.	Constraints	Garrett score	Garrett ranking
1.	Excessive rain	37.40	2
2.	Weed infestation	22.63	4
3.	Labour unavailability	14.63	5
4.	High labour cost	39.86	1
5.	Drought	28.10	3
6.	Storage problems	5.73	9
7.	Marketing problem	7.70	7
8.	Pest and diseases	14.53	6
9.	Trasportation, drying and threshing problems	6.43	8

4.1.1.1 *Labour cost and labour unavailability*

As per Garrett score maximum value was obtained for labour cost (39.86). In Kerala sesame is grown mostly in summer rice fallows. Properly tilled land is required for good germination of sesame seeds. Land preparation is a labour intensive operation and it requires skilled labour for operation of tractors and for obtaining a proper tillage of the field. Further intercultural operations, harvesting, transportation of harvested bundles, drying and winnowing also depends on labour availability. Lack of mechanization and unavailability of labour, which is ranked as fifth constraint as the farmers have to depend on labourers from other states which also contribute to additional labour cost.

4.1.1.2 *Excessive rain fall*

Data presented in Table 1 revealed that waterlogging associated with excessive rain fall was the second constraint of sesame production with the score of 37.40. Sesame often falls prey to unpredictable rains. According to farmer's response, excessive rain fall caused waterlogging in the field for 3-4 days, which resulted in decay of plants. Lodging of plants, yellowing of leaves followed by drying of plants were the major problems of waterlogging. It resulted in delayed harvesting, decay of harvested bundles, problems of drying and chaffy seeds. Waterlogging resulted in delayed and poor germination. The intermittent rain fall also resulted in non-uniform maturity of pods.

4.1.1.3 *Drought*

Drought associated with unavailability of rain fall is the 3rd major constraint reported by sesame farmers with a score of 28.10 (table 2). The consequences of drought faced by farmers are poor, delayed and non-uniform germination, poor growth and drying of plants.

4.1.1.4 *Weed infestation*

Weed infestation is the fourth ranked constraint in sesame production. *Cleome viscosa*, *Cynodon dactylon* and *Melochia corchorifolia* are the major weeds as per the

farmer's response. Among these *Melochia corchorifolia* has become a major menace in the sesame growing belt of Alappuzha and Kollam districts.

4.1.1.5 *Pests and diseases*

The sixth major constraint faced by farmers is the problems due to pest and diseases (14.63). Parrot attack is the main pest problem in sesame cultivation. A pandemonium of parrot attacks sesame at maturity stage, greatly troubling sesame growers. Phyllody caused by phytoplasma which makes the plants partially or entirely sterile at maturity stage is the minor disease observed.

4.1.1.6 *Marketing problem*

For large scale sesame growers, lack of market is a big problem which was ranked 7th. Most of the farmers sell sesame to nearby houses and local markets.

4.1.1.7 *Transportation, drying and threshing problem*

After harvest, transportation of bundles to drying yards is also a constraint (Garrett score 6.43). This is a problem for those who cultivate sesame in remote area. Others who cultivate in homesteads, lack of sufficient land area for sun drying of the bundles often causes problem. Lack of mechanization for drying and threshing is an added difficulty.

4.1.1.8 *Storage problem*

Storage pest (Garrett score 5.73) causes damage to sesame seeds, but it is a minor constraint which was ranked 9th.

4.2.1 SCREENING OF SESAME GENOTYPES FOR WATERLOGGING TOLERANCE

Fifteen sesame genotypes were screened for tolerance to waterlogging at both vegetative (20 DAS) and flowering (40 DAS) stage separately. The morpho-physiological and biochemical parameters were estimated in waterlogged and non-waterlogged (control) conditions and the means of both conditions (waterlogged and control) were analyzed using t- test. The percent change over control was also estimated. The finding are detailed below.

4.2.1.1 Morphological observations

4.2.1.1.1 Survival percentage (%)

The survival percentage of sesame genotypes waterlogged at vegetative and flowering stages are given in Table 7. At vegetative stage highest survival was recorded in *S. malabaricum* (100 %) followed by Ayali (77.33 %), SVPR1 (76.00 %), Thilarani (73.96 %) which were statistically on par. The lowest was recorded in CO 1 (30.50%) and Thilatara (35.67 %). When waterlogging was imposed at reproductive stage all varieties except CO1 (65.15 %) recorded 100 % survival.

4.2.1.1.2. Plant height (cm)

Plant height of sesame genotypes at vegetative stage (control) was varied from 39.00 cm to 71.75 cm as shown in Table 8 (control). Lowest plant height was recorded in *Sesamum malabaricum* (39.00 cm) followed by AT231 (58.50 cm). All other genotypes had higher and statistically similar plant heights.

Imposition of waterlogging at vegetative stage reduced the plant height of sesame genotypes as evident by the t- test as the t- statistics (5.058) was higher than t table value (2.048). Under waterlogged condition, 9 genotypes (Thilarani, Ayali, TMV7, SVPR1, TMV5, TMV 6, Thilak, Kayamkulam 1 and TMV3) recorded statistically higher and similar values. *Sesame malabaricum* (36.00 cm) recorded the lowest plant height. Percentage reduction in plant height over control ranged from 7.69 per cent in *Sesame malabaricum* to 31.23 per cent in CO1 which is the highest (Table 8).

Table7. Survival percentage of sesame genotypes under waterlogged and control condition

Sl. No	Genotypes	Survival percentage % (Vegetative stage)		Survival percentage % (Flowering stage)	
		Waterlogged	Control	Waterlogged	Control
1	TMV6	48.50^{hi} (0.770)	100	100.00^{a} (1.570)	100
2	CO1	30.50^{l} (0.585)	100	65.15^{b} (0.940)	100
3	GT 10	55.23^{g} (0.838)	100	100.00^{a} (1.570)	100
4	TMV 7	68.21 ^{ef} (0.972)	100	100.00a (1.570)	100
5	Thilarani	73.96^{bcd} (1.035)	100	100.00^{a} (1.570)	100
6	TMV4	43.22^{ij} (0.717)	100	100.00^{a} (1.570)	100
7	Kayamkulam 1	65.00^{f} (0.938)	100	100.00^{a} (1.570)	100
8	Thilatara	35.67^{kl} (0.640)	100	100.00^{a} (1.570)	100
9	Ayali	77.33^{b} (1.075)	100	100.00^{a} (1.570)	100
10	Thilak	71.50^{cde} (1.008)	100	100.00^{a} (1.570)	100
11	SVPR1	76.00^{bc} (1.059)	100	100.00^{a} (1.570)	100
12	TMV3	49.67^{gh} (0.782)	100	100.00^{a} (1.570)	100
13	AT 231	38.33^{jk} (0.668)	100	100.00^{a} (1.570)	100
14	TMV5	69.08^{def} (0.981)	100	100.00^{a} (1.570)	100
15	*S. malabaricum*	100.00^{a} (1.571)	100	100.00^{a} (1.570)	100
	CD (0.05)	0.057	NS	0.006	NS

Values in parenthesis indicate arc sin transformation

Table 8. Plant height (cm) of sesame varieties waterlogged at vegetative stage

Sl. No	Genotypes	Plant height (cm)		Percentage reduction over control
		Waterlogged	Control	
1	Kayamkulam 1	53.50^{abc}	67.00^{ab}	20.15
2	Thilarani	58.00^{a}	69.00^{ab}	15.94
3	Thilatara	44.50^{e}	64.50^{ab}	31.01
4	CO 1	43.50^{e}	63.25^{ab}	31.23
5	Thilak	54.50^{abc}	66.75^{ab}	18.35
6	TMV3	53.00^{abc}	64.25^{ab}	17.51
7	TVM 4	51.50^{bcd}	63.75^{ab}	19.22
8	TMV5	56.50^{ab}	70.50^{a}	19.86
9	TMV 6	54.50^{abc}	71.75^{a}	24.04
10	TMV7	57.00^{ab}	71.25^{a}	20.00
11	Ayali	57.50^{a}	70.00^{a}	17.86
12	SVPR 1	56.67^{ab}	70.00^{a}	19.05
13	AT 231	44.00^{de}	58.50^{b}	24.79
14	*S. malabaricum*	36.00^{f}	39.00^{c}	7.69
15	GT 10	49.33^{cde}	64.00^{ab}	22.92
	Mean	51.33	64.90	
	CD (0.05)	**5.982**	**11.107**	

t-stat- 5.058 **t table (0.05)- 2.048**

Table 9. Plant height (cm) of sesame genotypes waterlogged at flowering stage

Sl. no	Genotypes	Plant height (cm)		Percentage reduction over control
		Waterlogged	Control	
1	TMV6	62.33^{fg}	69.67^{fg}	10.32
2	CO1	72.33^{bcde}	91.67^{b}	21.31
3	GT 10	76.00^{bc}	85.33^{bc}	10.88
4	TMV 7	76.67^{b}	87.33^{bc}	10.75
5	Thilarani	73.33^{bcd}	80.67^{cde}	9.06
6	TMV4	67.67^{bcdef}	81.67^{cd}	16.53
7	Kayamkulam 1	67.33^{bcdef}	74.33^{def}	8.74
8	Thilatara	69.67^{bcdef}	85.67^{bc}	19.12
9	Ayali	66.67^{cdef}	72.33^{ef}	7.87
10	Thilak	63.00^{efg}	70.67^{fg}	10.82
11	SVPR1	69.33^{bcdef}	76.67^{def}	9.46
12	TMV3	64.33^{defg}	74.00^{def}	13.07
13	AT 231	55.67^{g}	62.67^{g}	10.80
14	TMV5	73.00^{bcd}	81.33^{cd}	9.75
15	*S. malabaricum*	88.33^{a}	107.67^{a}	17.72
	Mean	69.71	80.11	
	CD (0.05)	**9.384**	**8.362**	

t stat – 3.037 t table (0.05)- 2.048

Plant height of sesame genotypes at flowering stage is given in Table 9. In control condition, plant height ranged from 62.67 to 107.67 cm. The varieties AT 231(62.67 cm), TMV6 (69.67 cm), Thilak (70.67 cm), Ayali (72.33 cm), TMV3 (74.00 cm), Kayamkulam 1 (74.33 cm) and SVPR1 (76.67 cm) recorded lower plant height while *S. malabaricum* (107.67 cm) recorded the highest.

Waterlogging at flowering stage resulted in reduction of plant height as indicated by the higher t statistics (3.037) (Table 9). Under waterlogged condition highest plant height was maintained by *S. malabaricum* (88.33 cm). Lower plant height was recorded in AT231 (55.67 cm), TMV6 (62.33 cm), Thilak (63.00 cm) and TMV3 (64.33 cm). The reduction in plant height in sesame genotypes were ranged from 7.87 per cent in Ayali to 21.31 per cent in CO 1.

4.2.1.1.3. Root length (cm)

The root length of sesame varieties at vegetative stage is depicted in Table 10. Under control condition, the root length varied from 2.30 to 5.40 cm. Twelve genotypes (Thilak, Ayali, *S. malabaricum*, Kayamkulam 1, TMV5, TMV3, Thilarani, CO1, TMV6, TMV7, Thilatara, AT 231) recorded higher root length while three genotypes *viz*., TMV4, GT10 and SVPR1 recorded comparatively lower root length.

Since the t statistics (1.458) is lesser than t table value (2.048) there was no significant difference between overall means in root length of control and waterlogging treatment (Table 10).

The root length of sesame genotypes waterlogged at flowering stage are given in Table 11.The root length of sesame varieties varied from 6.33 cm to 10 cm at flowering stage. TMV5 (10.00cm), Thilatara (9.50 cm), TMV4 (9.33cm) and Ayali (8.83 cm) recorded highest root length. Lowest root length was recorded in TMV3 (6.33 cm), SVPR1 (6.5 cm), GT10 (7.23 cm), Kayamkulam 1 (7.33 cm), TMV7 (7.50 cm) and AT231 (7.50 cm).

During waterlogging at flowering stage overall root length of varieties was declined as indicated by the higher t statistics (6.224). Percent reduction in root length was lowest for TMV6 (10.55%) whereas highest for SVPR1 (43.20%) (Table 11).

Table 10. Root length (cm) of sesame genotypes waterlogged at vegetative stage

Sl. no	Genotypes	Vegetative stage	
		Waterlogged	Control
1	TMV6	4.00^{abc}	4.25^{ab}
2	CO1	4.00^{abc}	4.67^{ab}
3	GT 10	2.88^{cd}	3.40^{bc}
4	TMV 7	3.88^{abc}	4.11^{ab}
5	Thilarani	4.63^{ab}	4.93^{ab}
6	TMV4	1.93^{d}	2.30^{c}
7	Kayamkulam 1	4.75^{a}	5.20^{a}
8	Thilatara	3.5^{abcd}	4.00^{ab}
9	Ayali	5.00^{a}	5.35^{a}
10	Thilak	4.50^{abc}	5.40^{a}
11	SVPR1	3.00^{bcd}	3.50^{bc}
12	TMV3	4.75^{a}	5.05^{ab}
13	AT 231	3.38^{abcd}	3.83^{abc}
14	TMV5	4.50^{abc}	5.17^{a}
15	*S. malabaricum*	4.63^{ab}	5.25^{a}
	Mean	3.955	4.427
	CD (0.05)	**1.733**	**1.653**

t statistics (1.458) **t table (0.05)- 2.048**

Table 11. Root length (cm) of sesame varieties waterlogged at flowering stage

Sl. no	Genotypes	Flowering stage		Percentage reduction over control
		Waterlogged	Control	
1	TMV6	6.67^{a}	7.668^{cdef}	10.55
2	CO1	5.67^{abc}	8.67^{bcd}	33.97
3	GT 10	4.50^{cde}	7.23^{efg}	37.82
4	TMV 7	6.00^{ab}	7.50^{defg}	20.06
5	Thilarani	6.33^{ab}	7.67^{cdef}	14.07
6	TMV4	6.17^{ab}	9.33^{ab}	33.47
7	Kayamkulam 1	5.83^{ab}	7.33^{efg}	21.26
8	Thilatara	5.67^{abc}	9.50^{ab}	40.05
9	Ayali	4.33^{de}	8.83^{abc}	50.55
10	Thilak	5.83^{ab}	7.83^{cde}	25.66
11	SVPR1	3.67^{e}	6.50^{fg}	43.20
12	TMV3	4.50^{cde}	6.33^{g}	27.64
13	AT 231	5.17^{bcd}	7.50^{defg}	31.51
14	TMV5	6.17^{ab}	10.00^{a}	38.15
15	*S. malabaricum*	6.50a	7.67^{cdef}	14.74
	Mean	5.53	7.97	
	CD (0.05)	**1.288**	**1.302**	

t- statistics: 6.224 **t table value (0.05): 2.048**

Under waterlogged condition at flowering stage, TMV 6 (6.67 cm), *S. malabaricum* (6.50 cm), Thilarani (6.33 cm), TMV5 (6.17 cm), TMV4 (6.17 cm), TMV7 (6.00cm), Kayamkulam 1 (5.83 cm), Thilak (5.83 cm), Thilatara (5.67 cm) and CO1 (5.67 cm) recorded highest root length whereas, SVPR1 (3.7 cm), Ayali (4.33 cm), TMV3 (4.50 cm) and GT 10 (4.50 cm) recorded the lowest root length (Table 11).

4.2.1.1.4. Root number

Root number of sesame genotypes at vegetative stage are given in Table 12. Under control condition no statistical difference was observed among genotypes for the root number. The treatment means of control and waterlogged condition was not statistically different as indicated by lower t statistics (1.852).

The percentage change in root number over control indicated that, in the genotypes Thilatara, AT231 and CO1 root number was decreased during waterlogging, while in all other varieties increase in root number was observed with highest increase being observed in Ayali (24.22 %) as shown in Table 12. Under stress condition, Ayali (29.25), Thilarani (28.25), *Sesamum malabaricum*, (27.50) TMV6 (26.00), TMV5 (24.75), Kayamkulam 1 (24.75), SVPR 1(24.25) and GT10 (23.50) recorded highest root number and the lowest root number was observed in AT231 (15.00) which is statistically on par with Thilatara (17.25) and CO1 (18.75) (Table 12).

Root number of sesame genotypes waterlogged at flowering stage are given in Table 13 (control). Under control condition the root number varied significantly. *S. malabaricum* (96.00) recorded the highest whereas lowest root number was recorded in AT231 (45.00), TMV 6 (45.00), TMV 4 (47.00), MV 3 (49.33), GT 10 (50.33), TMV 7(50.67) and SVPR 1 (51.00). No significant variation in root number was observed among waterlogged and control plants as lower t statistic indicated (1.695).

Table 12. Root number of sesame genotypes waterlogged at vegetative stage

Sl. No	Genotypes	Vegetative stage		Percentage change over control
		Waterlogged	Control	
1	TMV6	26.00^{abc}	21.67	16.67
2	CO1	18.75^{def}	24.00	-28.00
3	GT 10	23.50^{abcd}	21.33	9.22
4	TMV 7	22.75^{bcde}	18.67	17.95
5	Thilarani	28.25^{ab}	22.00	22.12
6	TMV4	22.23^{cde}	20.92	5.89
7	Kayamkulam 1	24.75^{abc}	21.58	12.79
8	Thilatara	17.25^{ef}	23.67	-37.20
9	Ayali	29.25^{a}	22.17	24.22
10	Thilak	22.00^{cde}	21.00	4.55
11	SVPR1	24.25^{abcd}	20.33	16.15
12	TMV3	22.50^{bcde}	19.33	14.07
13	AT 231	15.00^{f}	20.33	-35.56
14	TMV5	24.75^{abc}	19.67	20.54
15	*S. malabaricum*	27.50^{abc}	21.75	20.91
	Mean	23.249	21.228	
	CD (0.05)	**5.869**	**NS**	

t-statistics- 1.320 **t table (0.05)- 2.048**

Table 13. Root number of sesame genotypes waterlogged at flowering stage

Sl. no	Genotypes	Flowering stage	
		Waterlogged	Control
1	TMV6	40.67^{fg}	45.00^{g}
2	CO1	51.00^{bcde}	68.00^{b}
3	GT 10	46.67^{def}	50.33^{efg}
4	TMV 7	40.33^{g}	50.67^{efg}
5	Thilarani	53.67^{bc}	59.33^{c}
6	TMV4	45.33^{efg}	47.00^{fg}
7	Kayamkulam 1	57.00^{b}	60.00^{c}
8	Thilatara	51.67^{bcd}	55.33^{cde}
9	Ayali	53.33^{bc}	58.67^{cd}
10	Thilak	55.33^{b}	56.67^{cde}
11	SVPR1	48.33^{cde}	51.00^{defg}
12	TMV3	39.67^{g}	49.33^{efg}
13	AT 231	33.00^{h}	45.00^{g}
14	TMV5	49.00^{cde}	53.33^{cdef}
15	*S. malabaricum*	75.67^{a}	92.67^{a}
	Mean	49.38	56.16
	CD (0.05)	**6.245**	**7.852**

t-statistics- 1.695 **t table (0.05)- 2.048**

4.2.1.1.5. Root dry weight (g)

Root dry weight was varied among genotypes in vegetative stage as given in Table 14. TMV3 (0.047), Thilatara (0.046), Thilak (0.045), CO1 (.044), SVPR1 90.042) and TMV4 (0.041) recorded higher root dry weight under control.

The treatment means of control and waterlogged conditions were statistically on par indicated by the lower t statistics (0.959). Reduction in root dry weight was recorded in genotypes, CO1, TMV4, Thilatara, Thilak, TMV3 and AT231 whereas other genotypes recorded increase in root dry weight over control (Table 14).

Under waterlogged condition, TMV5 (0.057g), Thilarani (0.056g), Ayali (0.056g), GT 10 (0.050g) and SVPR1 (0.048g) recorded higher root dry weight whereas CO1 (0.026g), Thilatara (0.027g), AT231 (0.028g) and TMV4 (0.030g) recorded lower root dry weight (Table 14).

Root dry weight was varied among genotypes at flowering stage (control) as given in Table 15. *S. malabaricum* recorded highest root dry weight, all others recorded lower values. The treatment means of control and waterlogged conditions were statistically on par indicated by the lower t statistics (0.621).

Table 14. Root dry weight (g) of sesame genotypes waterlogged at vegetative stage

Sl. No	Genotypes	Vegetative stage		Percentage change over control
		Waterlogged	Control	
1	TMV6	0.043^{bc}	0.037^{cde}	16.07
2	CO1	0.026^{e}	0.044^{abc}	-41.67
3	GT 10	0.050^{ab}	0.034^{de}	46.08
4	TMV 7	0.045^{bc}	0.033^{ef}	37.44
5	Thilarani	0.056^{a}	0.037^{cde}	50.45
6	TMV4	0.030^{de}	0.041^{abcd}	-27.42
7	Kayamkulam 1	0.045^{bc}	0.038^{bcde}	16.52
8	Thilatara	0.027^{e}	0.046^{ab}	-42.03
9	Ayali	0.056^{a}	0.037^{cde}	50.45
10	Thilak	0.042^{bc}	0.045^{abc}	-7.04
11	SVPR1	0.048^{ab}	0.042^{a}	15.20
12	TMV3	0.037^{cd}	0.047^{abcd}	-21.13
13	AT 231	0.028^{e}	0.038^{bcde}	-25.44
14	TMV5	0.057^{a}	0.039^{bcde}	47.41
15	*S. malabaricum*	0.038^{cd}	0.025^{f}	50.67
	CD (0.05)	**0.009**	**0.008**	

t-stat- 0.959 **t table (0.05)- 2.048**

Table 15. Root dry weight (g) of sesame genotypes waterlogged at flowering stage

Sl. no	Genotypes	Flowering stage	
		Waterlogged	Control
1	TMV6	0.141^{cd}	0.207^{bc}
2	CO1	0.298^{b}	0.418^{b}
3	GT 10	0.233^{bcd}	0.257^{bc}
4	TMV 7	0.192^{bcd}	0.243^{bc}
5	Thilarani	0.243^{bc}	0.322^{bc}
6	TMV4	0.107^{d}	0.162^{c}
7	K1	0.215^{bcd}	0.257^{bc}
8	Thilatara	0.140^{cd}	0.195^{bc}
9	Ayali	0.206^{bcd}	0.241^{bc}
10	Thilak	0.164^{cd}	0.221^{bc}
11	SVPR1	0.309^{b}	0.331^{bc}
12	TMV3	0.191^{bcd}	0.228^{bc}
13	AT 231	0.135^{cd}	0.164^{c}
14	TMV5	0.198^{bcd}	0.268^{bc}
15	*S. malabaricum*	1.510^{a}	2.288^{a}
	CD (0.05)	**0.129**	**0.251**

t-stat- 0.621 **t table (0.05)- 2.048**

4.2.1.1.6. Shoot dry weight (g)

Significant variation in shoot dry weight was observed among genotypes as shown in Table 16 (control). TMV7 (0.603g), TMV5 (0.603g), Thilarani (0.03g), TMV6 (0.600g), SVPR1 (0.600g), Ayali (0.590g), TMV4 (0.560g) and Kayamkulam1 (0.557g) recorded highest shoot dry weight while *Sesamum malabaricum* (0.233g) recorded the lowest.

Higher t statistics (6.321) indicated that waterlogging reduced the shoot dry weight of sesame genotypes (Table 16). Under waterlogged condition, Thilarani (0.447g), Ayali (0.433g), TMV5 (0.423g) and SVPR1 (0.410g) recorded the highest shoot dry weight. *Sesamum malabaricum* (0.217g), AT231 (0.240g), CO1 (0.243g) and Thilatara (0.253g) recorded the lowest. Lowest percent reduction in shoot dry weight was observed in *Sesamum malabaricum* (7.14%) and highest in CO1 (53.80%) (Table 16).

The variation in shoot dry weight of sesame at reproductive stage are given in Table 17 (control). The shoot dry weight was varied from 1.34 g to 4.70 g. The lowest shoot dry weight was recorded in Thilak, AT231, Ayali, GT 10, Kayamkulam 1, TMV5, TMV7, TMV4, TMV6 and Thilarani. The genotypes which recorded highest shoot dry weight was *S. malabaricum.*

Waterlogging decreased the shoot dry weight of sesame varieties as per the t statistics (3.261). Under waterlogged condition, *S. malabaricum* (3.167g) recorded highest shoot dry weight followed by SVPR 1, CO1 (1.763 g) and TMV3 (1.670g). All others recorded lower shoot dry weight (Table 17).

Thilatara recorded highest per cent reduction of 58.10% in shoot dry weight whereas TMV 5 recorded the lowest reduction of 20.20 % (Table 17).

Table 16. Shoot dry weight (g) of sesame genotypes waterlogged at vegetative stage

Sl. no	Genotypes	Vegetative stage		Percentage reduction over control
		Waterlogged	Control	
1	TMV6	0.337^{d}	0.600^{a}	43.89
2	CO1	0.243^{e}	0.527^{c}	53.80
3	GT 10	0.330^{d}	0.540^{bc}	38.89
4	TMV 7	0.370^{cd}	0.603^{a}	38.67
5	Thilarani	0.447^{a}	0.603^{a}	25.93
6	TMV4	0.340^{d}	0.560^{abc}	39.29
7	Kayamkulam 1	0.377^{bcd}	0.557^{abc}	32.34
8	Thilatara	0.253^{e}	0.523^{c}	51.53
9	Ayali	0.433^{a}	0.590^{ab}	26.55
10	Thilak	0.363^{cd}	0.537^{bc}	32.30
11	SVPR1	0.410^{abc}	0.600^{a}	31.67
12	TMV3	0.350^{d}	0.521^{cd}	32.78
13	AT 231	0.240^{e}	0.463^{d}	48.20
14	TMV5	0.423^{ab}	0.603^{a}	29.83
15	*S. malabaricum*	0.217^{e}	0.233^{e}	7.14
	CD (0.05)	**0.051**	**0.059**	

t statistics : 6.321 **t table (0.05)- 2.048**

Table 17. Shoot dry weight (g) of sesame genotypes waterlogged at flowering stage

Sl. No	Genotypes	Flowering stage		Percentage reduction over control
		Waterlogged	Control	
1	TMV6	1.002^{de}	2.26^{cde}	55.6
2	CO1	1.763^{bc}	3.28^{b}	46.3
3	GT 10	1.393^{cde}	1.79^{de}	22.2
4	TMV 7	1.147^{cde}	2.08^{cde}	44.9
5	Thilarani	1.442^{cde}	2.27^{cde}	36.5
6	TMV4	1.009^{de}	2.18^{cde}	53.8
7	Kayamkulam1	1.186^{cde}	1.97^{de}	39.8
8	Thilatara	1.220^{cde}	2.91^{bc}	58.1
9	Ayali	1.131^{cde}	1.55^{de}	27.2
10	Thilak	0.909^{e}	1.34^{e}	32.1
11	SVPR1	2.351^{b}	3.58^{b}	34.3
12	TMV3	1.670^{bcd}	2.32^{cd}	28.1
13	AT 231	1.013^{de}	1.47^{de}	31.0
14	TMV5	1.581^{cde}	1.98^{cde}	20.2
15	*S. malabaricum*	3.167^{a}	4.70^{a}	32.6
	CD (0.05)	**0.733**	**0.936**	

t statistics: 3.261 **t table (0.05)- 2.048**

4.2.1.2 Biochemical observations

4.2.1.2.1. Chlorophyll content (mg g^{-1})

Chlorophyll content of sesame genotypes waterlogged at vegetative stage and control condition are given in Table 18. At vegetative stage no significant variation was observed among genotypes for the chlorophyll content under control condition (Table 18). Waterlogging significantly reduced the chlorophyll content of sesame genotypes as evident in the t test. Since t-statistic for chlorophyll content (18.760) after waterlogging was more than table t value (2.048), there was significant difference between waterlogging treatment and control for chlorophyll content. Under waterlogged condition, the genotypes which recorded highest chlorophyll content were *Sesamum malabaricum* (1.40), TMV5 (1.35 mg g^{-1}), Thilarani (1.33 mg g^{-1}), TMV7 (1.30 mg g^{-1}) and Ayali (1.30 mg g^{-1}) whereas lowest was seen Thilatara (1.11 mg g^{-1}), AT231 (1.14 mg g^{-1}), TMV4 (1.16 mg g^{-1}) and CO1 (1.18 mg g^{-1}) (Table 18).

Chlorophyll content was reduced with waterlogging in all varieties. Percentage reduction in chlorophyll content over control indicated that (Table 18), Thilatara (32.27%) recorded highest per cent reduction whereas, *Sesamum malabaricum* (18.04 %) and Thilarani (18.56%) recorded the least reduction.

As in the case of vegetative stage, at flowering stage also chlorophyll content was varied among genotypes (Table 19). CO1 (1.22 mg g^{-1}), AT231 (1.22 mg g^{-1}), GT 10 (1.21 mg g^{-1}) and *S. malabaricum* (1.20 mg g^{-1}) recorded highest, whereas Thilarani (1.07 mg g^{-1}), Kayamkulam 1 (1.08 mg g^{-1}), Ayali (1.09 mg g^{-1}) and TMV4 (1.09 mg g^{-1}) recorded the lowest values of chlorophyll content. Waterlogging significantly reduced the chlorophyll content in all genotypes. Since t-statistics (4.398) of chlorophyll content after waterlogging was more than table t value (2.048), there was significant difference among control and waterlogging treatment. The 11 genotypes which recorded highest chlorophyll content under waterlogged condition were *S. malabaricum* (1.09 mg g^{-1}), AT 231 (1.09 mg g^{-1}), GT 10 (1.07 mg g^{-1}), TMV6 (1.06 mg g^{-1}), CO1 (1.05 mg g^{-1}), TMV3 (1.05 mg g^{-1}), Ayali (1.03 mg g^{-1}), TMV5 (1.03 mg g^{-1}), TMV 4 (1.00 mg g^{-1}), Kayamkulam 1 (1.00 mg g^{-1}) and TMV7 (0.99 mg g^{-1}) (Table 19). The other four genotypes, Thilatara (0.91 mg g^{-1}), SVPR1 (0.97 mg g^{-1}), Thilak

Table 18. Chlorophyll content (mgg^{-1}) of sesame genotypes waterlogged at vegetative stage

Sl. No	Genotypes	Chlorophyll content (mgg^{-1})		Percentage reduction over control
		Waterlogged	Control	
1	TMV6	1.22^{defg}	1.63	25.24
2	CO1	1.18^{efgh}	1.64	27.84
3	GT 10	1.24^{cdefg}	1.70	26.34
4	TMV 7	1.30^{abcd}	1.66	21.44
5	Thilarani	1.33^{abc}	1.65	18.56
6	TMV4	1.16^{fgh}	1.69	31.38
7	Kayamkulam 1	1.27^{bcde}	1.67	23.88
8	Thilatara	1.11^{h}	1.63	32.27
9	Ayali	1.30^{abcd}	1.66	22.05
10	Thilak	1.29^{bcd}	1.70	23.83
11	SVPR1	1.23^{cdefg}	1.66	25.61
12	TMV3	1.26^{bcdef}	1.69	24.17
13	AT 231	1.14^{gh}	1.63	30.05
14	TMV5	1.35^{ab}	1.69	20.27
15	*S. malabaricum*	1.40^{a}	1.71	18.04
	Mean	1.25	1.67	
	CD (0.05)	**0.106**	**NS**	

t stat – 18.760 t table (0.05)- 2.048

Table 19. Chlorophyll content (mgg^{-1}) of sesame genotypes waterlogged at flowering stage

Sl. no	Genotypes	Flowering stage		Percentage reduction over control
		Waterlogged	Control	
1	SVPR 1	0.97^{bcd}	1.14^{bc}	14.79
2	CO1	1.05^{ab}	1.22^{a}	13.89
3	GT10	1.07^{ab}	1.21^{a}	12.09
4	AT231	1.09^{a}	1.22^{a}	11.20
5	Ayali	1.03^{abc}	1.09^{cde}	5.43
6	Thilatara	0.91^{d}	0.96^{f}	4.93
7	Thilarani	0.97^{bcd}	1.07^{de}	9.41
8	Kayamkulam 1	1.00^{abcd}	1.08^{cde}	7.36
9	Thilak	0.94^{cd}	1.04^{e}	8.83
10	TMV 7	0.99^{abcd}	1.08^{cde}	7.73
11	TMV 6	1.06^{ab}	1.13^{cd}	6.66
12	TMV 5	1.03^{abc}	1.13^{cd}	8.83
13	TMV 4	1.00^{abcd}	1.09^{cde}	8.27
14	TMV 3	1.05^{ab}	1.14^{bc}	7.31
15	*S.malabaricum*	1.09^{a}	1.20^{ab}	9.09
	CD (0.05)	**0.102**	**0.066**	

t stat – 4.398 t table (0.05)- 2.048

(0.94 mg g^{-1}) and Thilarani (0.97 mg g^{-1}) which recorded lowest chlorophyll content was statistically on par with TMV7, Kayamkualm 1 and TMV 4. SVPR 1 (14.79%) recorded highest per cent reduction in chlorophyll content while Thilatara (4.93) recorded the lowest (Table 19).

4.2.1.2.2. MDA content (nmol g^{-1})

The MDA content in sesame genotypes at vegetative stage ranged from 2.24 nmol g^{-1} to 5.25 nmol g^{-1} (Table 20). The genotypes TMV3 (2.24 nmol g^{-1}), *S. malabaricum* (2.28 nmol g^{-1}) TMV5 (2.33 nmol g^{-1}), TMV7 (2.93 nmol g^{-1}), AT231 (3.05 nmol g^{-1}), SVPR 1 (3.17 nmol g^{-1}), TMV4 (3.36 nmol g^{-1}) recorded the lowest whereas, CO 1 (5.25 nmol g^{-1}), Thilatara (5.13 nmol g^{-1}), GT10 (4.27 nmol g^{-1}), TMV6 (4.17 nmol g^{-1}) recorded the highest MDA content.

Waterlogging resulted in an increase in MDA production in all sesame genotypes as indicated by the higher t statistics (4.176) than t table value (2.048). Thilatara (11.08 nmol g^{-1}) and CO1 (10.98 nmol g^{-1}) recorded highest whereas *S. malabaricum* (3.18 nmol g^{-1}) and TMV5 (3.61 nmol g^{-1}) recorded the lowest MDA content under waterlogged condition (Table 20).

Significant variation in percentage increase in MDA content over control in different genotypes were observed as seen in Table 20. Higher per cent increase was observed in the genotype Thilatara (116.1%) and lowest in *S. malabaricum* (39.9 %).

MDA production was varied among genotypes from 0.55 nmol g^{-1} to 1.03 nmol g^{-1} under flowering stage (Table 21). Thilatara (1.03 nmol g^{-1}), TMV7 (1.02 nmol g^{-1}) and TMV6 (0.99 nmol g^{-1}) and SVPR1 (0.92 nmol g^{-1}) recorded higher MDA production whereas Ayali (3.78 nmol g^{-1}), GT10 (0.59 nmol g^{-1}), AT231 (3.05 nmol g^{-1}) and Thilak (3.55 nmol g^{-1}) recorded lowest MDA content.

The t-statistics (3.192) of MDA content under waterlogging is higher than table t value (2.048), indicated significant difference between waterlogged and control condition at flowering stage (Table 21). Thilatara (1.33 nmol g^{-1}), TMV7 (1.30 nmol g^{-1}), TMV6 (1.25 nmol g^{-1}), TMV3 (1.12 nmol g^{-1}), Thilarani (1.10 nmol g^{-1}), CO1 (1.07 nmol g^{-1}),

Table 20. MDA content of sesame genotypes waterlogged at vegetative stage

Sl. No	Varieties	MDA content (nmol g^{-1})		Percentage increase over control
		Waterlogged	Control	
1	TMV6	6.57^{c}	4.17^{abc}	57.6
2	CO1	10.98^{a}	5.25^{a}	109.2
3	GT 10	8.37^{b}	4.27^{ab}	96.2
4	TMV 7	5.44^{d}	2.93^{cde}	85.3
5	Thilarani	5.41^{d}	3.64^{bc}	48.6
6	TMV4	6.32^{cd}	3.36^{bcde}	88.3
7	Kayamkulam 1	5.44^{d}	3.75^{bc}	45.1
8	Thilatara	11.08^{a}	5.13^{a}	116.1
9	Ayali	5.40^{d}	3.78^{bc}	42.7
10	Thilak	5.87^{cd}	3.55^{bcd}	65.4
11	SVPR1	5.53^{d}	3.17^{bcde}	74.7
12	TMV3	4.18^{e}	2.24^{e}	86.4
13	AT 231	5.75^{cd}	3.05^{bcde}	88.5
14	TMV5	3.61^{ef}	2.33^{de}	55.4
15	*S. malabaricum*	3.18^{f}	2.28^{e}	39.9
	Mean	6.209	3.527	
	CD (0.05)	**0.993**	**1.254**	

t stat – 4.176 t table (0.05)- 2.048

Table 21. MDA content of sesame genotypes waterlogged at flowering stage

Sl. No	Genotypes	MDA content (nmol g^{-1})		Percentage increase over control
		Waterlogged	Control	
1	SVPR 1	1.07^{abcd}	0.92^{abc}	16.44
2	CO1	1.07^{abcd}	0.79^{de}	34.65
3	GT10	0.706^{f}	0.59^{fg}	18.11
4	AT231	0.77^{ef}	0.60^{fg}	28.84
5	Ayali	0.72^{ef}	0.55^{g}	31.10
6	Thilatara	1.33^{a}	1.03^{a}	29.64
7	Thilarani	1.10^{abc}	0.89^{bcd}	24.40
8	Kayamkulam 1	0.87^{cdef}	0.70^{ef}	23.37
9	Thilak	0.80^{def}	0.62^{fg}	29.14
10	TMV 7	1.30^{a}	1.02^{a}	26.83
11	TMV 6	1.25^{ab}	0.99^{ab}	26.55
12	TMV 5	1.07^{abcd}	0.89^{bcd}	20.36
13	TMV 4	1.00^{bcde}	0.77^{de}	29.89
14	TMV 3	1.12^{abc}	0.86^{bcd}	29.91
15	*S. malabaricum*	1.07^{abcd}	0.81^{cde}	32.51
	CD (0.05)	**0.293**	**0.129**	

t stat – 3.192 t table (0.05)- 2.048

SVPR1 (1.07 nmol g^{-1}), TMV5 (1.07 nmol g^{-1}), *S.malabaricum* (1.07 nmol g^{-1}) recorded the highest MDA content. GT10 (0.706 nmol g^{-1}), Ayali (072 nmol g^{-1}), AT231 (0.77 nmol g^{-1}), Thilak (0.80 nmol g^{-1}) and Kayamkulam 1 (0.87 nmol g^{-1}) recorded the lowest MDA content under waterlogged condition (Table 21).

The varieties showed significant variation in per cent increase in MDA content over control. CO 1 (34.65 %) recorded highest and SVPR 1 (16.44 %) recorded the lowest percentage increase (Table 21).

4.2.1.2.3. Catalase enzyme activity (µ mol of H_2O_2 utilized $g^{-1}min^{-1}$)

Sesame genotypes at vegetative stage showed statistically on par values of catalase enzyme activity (Table 22). In general waterlogging significantly increased the catalase enzyme activity as the t statistics (3.963) is higher than t table value (2.048). Catalase enzyme activity of four genotypes AT231, TMV4, CO1 and TMV6 were found to be decreasing under waterlogging in contrast to others. *S. malabaricum* (143.30 µ mol of H_2O_2 utilized $g^{-1}min^{-1}$) recorded highest catalase enzyme activity and TMV6 (75.02 µ mol of H_2O_2 utilized $g^{-1}min^{-1}$) recorded the lowest under waterlogging condition.

The catalase enzyme activity of sesame genotypes waterlogged at flowering stage are given in Table 23. The catalase enzyme activity in control and waterlogged treatments were found to be non-significant among genotypes (Table 23). However, the catalase activity has increased under waterlogged condition indicated by higher t-statistics (18.685). Variation in per cent increase in catalase enzyme activity ranged from 25 to 39 per cent.

4.2.1.2.4. Total soluble protein content (mg g^{-1})

Total soluble protein content was varied among varieties at vegetative stage with a range of 8.07 mg g^{-1} in CO 1 to 17.20 mg g^{-1} in TMV7 (Table 24). Waterlogging reduced the total soluble protein content as evident in higher t-statistics (3.715). Significant variation was observed under waterlogged condition. The genotypes, TMV 5 (12.17 mg g^{-1}), TMV7 (11.75 mg g^{-1}), Thilarani (11.67 mg g^{-1}), GT10 (10.49 mg g^{-1}) and Thilak (9.70 mg g^{-1}) recorded highest whereas CO1 (4.38 mg g^{-1}), TMV6 (4.50 mg

Table 22. Catalase enzyme activity of sesame genotypes waterlogged at vegetative stage

Sl. No	Genotypes	Catalase activity (µ mol of H_2O_2 utilized $g^{-1}min^{-1}$)		Percentage change over control
		Waterlogged	Control	
1	TMV6	75.02^{j}	78.75	-1.36
2	CO 1	89.67^{hi}	94.25	-2.77
3	GT 10	120.92^{def}	86.25	43.33
4	TMV 7	130.94^{bc}	91.75	43.61
5	Thilarani	136.15^{b}	98.25	38.89
6	TMV4	91.57^{h}	98.25	-6.74
7	Kayamkulam 1	120.46^{ef}	85.50	41.29
8	Thilatara	127.70^{cd}	92.00	39.08
9	Ayali	128.00^{cd}	90.50	42.29
10	Thilak	124.97^{cde}	93.00	37.08
11	SVPR 1	116.27^{f}	90.00	29.39
12	TMV3	94.67^{h}	81.25	19.79
13	AT 231	84.00^{i}	91.50	-8.14
14	TMV5	106.00^{g}	84.50	25.73
15	*S. malabaricum*	143.30^{a}	98.75	45.13
	CD (0.05)	**7.145**	**NS**	

t- stat- 3.963 t table (0.05)- 2.048

Table 23. Catalase enzyme activity of sesame genotypes waterlogged flowering stage

Sl. No	Varieties	Catalase activity (µ mol of H_2O_2 utilized $g^{-1}min^{-1}$)		Percentage increase over control
		Waterlogged	Control	
1	TMV6	135.0	106.7	26.54
2	CO1	137.8	110.0	25.26
3	GT 10	145.2	108.0	34.43
4	TMV 7	148.6	113.0	31.49
5	Thilarani	142.5	108.7	31.10
6	TMV4	147.3	109.7	34.32
7	Kayamkulam 1	149.5	107.3	39.26
8	Thilatara	140.5	106.0	32.56
9	Ayali	147.6	108.0	36.69
10	Thilak	131.8	102.3	28.80
11	SVPR1	146.8	111.4	31.72
12	TMV3	141.0	104.7	34.74
13	AT 231	130.8	101.8	28.48
14	TMV5	146.3	113.8	28.48
15	*S. malabaricum*	146.2	111.9	30.61
	CD (0.05)	**NS**	**NS**	

t stat – 18.685 t table (0.05)- 2.048

Table. 24. Total soluble protein content of sesame genotypes waterlogged at vegetative stage

Sl. No	Genotypes	Soluble protein content (mgg^{-1})		Percentage reduction over control
		Waterlogged	Control	
1	TMV6	4.50^{ef}	8.33^{e}	46.0
2	CO1	4.38^{f}	8.07^{e}	45.8
3	GT 10	10.49^{ab}	15.52^{ab}	32.4
4	TMV 7	11.75^{a}	17.20^{a}	31.7
5	Thilarani	11.67^{a}	15.00^{ab}	22.2
6	TMV4	4.50^{ef}	10.00^{de}	55.0
7	Kayamkulam 1	8.00^{bcd}	11.33^{cde}	29.4
8	Thilatara	7.17^{cde}	11.33^{cde}	36.8
9	Ayali	8.43^{bcd}	11.33^{cde}	25.6
10	Thilak	9.70^{abc}	12.33^{bcd}	21.4
11	SVPR1	6.67^{def}	8.83^{e}	24.5
12	TMV3	8.33^{bcd}	11.20^{de}	25.6
13	AT 231	6.75^{def}	11.33^{cde}	40.4
14	TMV5	12.17^{a}	14.67^{abc}	17.0
15	*S. malabaricum*	6.63^{def}	9.55^{de}	30.6
	CD (0.05)	**2.733**	**3.406**	

t-stat- 3.715 **t table (0.05)- 2.048**

Table 25. Total soluble protein content of sesame genotypes waterlogged at flowering stage

Sl. No	Genotypes	Soluble protein content (mgg^{-1})	
		Waterlogged	Control
1	SVPR 1	10.03	11.20
2	CO 1	16.67	32.11
3	GT 10	19.87	28.33
4	AT 231	14.00	20.00
5	Ayali	25.67	31.67
6	Thilatara	20.53	23.44
7	Thilarani	20.00	25.83
8	Kayamkulam 1	19.20	29.19
9	Thilak	32.33	37.67
10	TMV7	24.00	29.67
11	TMV6	13.33	16.33
12	TMV5	11.87	17.83
13	TMV4	3.83	5.90
14	TMV3	5.67	12.00
15	*S. malabaricum*	14.53	19.00

t stat- 1.945 **t table (0.05)- 2.048**

g^{-1}), TMV4 (4.50 mg g^{-1}), *S. malabaricum* (6.63 mg g^{-1}), SVPR 1 (6.67 mg g^{-1}) and AT231 (6.75 mg g^{-1}) recorded the lowest. Highest percent reduction was observed in TMV 4 (55 %) and lowest in TMV5 (17 %) (Table 24).

At flowering stage in control condition, total soluble protein content ranged from 3.67 mg g^{-1} (Thilak) to 5.90 mg g^{-1} (TMV 4). No significant change in soluble protein with waterlogging was observed as evident in the lower t- statistics (1.945) (Table 25).

4.2.1.2.5. Nitrate reductase enzyme (NRase) activity (µ moles of NO_2^- formed $g^{-1}hr^{-1}$)

NRase activity at vegetative stage was found to be statistically non-significant among different genotypes (Table 26). Waterlogging decreased the NRase activity in all sesame genotypes as indicated by higher t statistics (5.02). Under waterlogged condition, Thilarani (1066.67 µ moles of $NO_2^-g^{-1}hr^{-1}$), Kayamkulam 1 (1066.67 µ moles of $NO_2^-g^{-1}hr^{-1}$), Thilak (1006.67 µ moles of $NO_2^-g^{-1}hr^{-1}$), TMV3 (973.33 µ moles of $NO_2^-g^{-1}hr^{-1}$), TMV6 (933.33 µ moles of $NO_2^-g^{-1}hr^{-1}$), SVPR 1 (900.00 µ moles of $NO_2^-g^{-1}hr^{-1}$), GT10 (877.33 µ moles of $NO_2^-g^{-1}hr^{-1}$), TMV7 (876.67 µ moles of $NO_2^-g^{-1}hr^{-1}$), Ayali (800.00 µ moles of $NO_2^-g^{-1}hr^{-1}$) and TMV5 (766.67 µ moles of $NO_2^-g^{-1}hr^{-1}$) recorded highest NRase activity whereas, Thilatara (413.33 µ moles of $NO_2^-g^{-1}hr^{-1}$), CO 1 (518.67 µ moles of $NO_2^-g^{-1}hr^{-1}$), AT231 (528.67 µ moles of $NO_2^-g^{-1}hr^{-1}$) and TMV 4 (686.67 µ moles of $NO_2^-g^{-1}hr^{-1}$) recorded the lowest activity. NRase activity was declined by 49.68 per cent in Thilatara and 17.23 per cent in Ayali (table 26).

NRase activity of sesame genotypes at flowering stage was significantly varied (Table 27). Highest Nrase activity was observed in Thilarani (890.00 µ moles of $NO_2^-g^{-1}hr^{-1}$), Kayamkulam 1 (893.25 µ moles of $NO_2^-g^{-1}hr^{-1}$) and Thilak (816.67 µ moles of $NO_2^-g^{-1}hr^{-1}$). All other genotypes recorded statistically on par values.

Table 26. NRase activity of sesame genotypes waterlogged at vegetative stage

Sl. No	Genotypes	NRase activity (µ moles of NO_2^- formed $g^{-1}hr^{-1}$)		Percentage reduction over control
		Waterlogged	Control	
1	SVPR 1	900.00^{ab}	1416.67	27.45
2	CO1	518.67^{cd}	933.33	43.87
3	GT10	877.33^{ab}	1316.67	26.52
4	AT231	528.67^{cd}	1000.00	42.17
5	Ayali	800.00^{abc}	966.67	17.23
6	Thilatara	413.33^{d}	833.33	49.68
7	Thilarani	1066.67^{a}	1385.33	22.18
8	Kayamkulam 1	1066.67^{a}	1400.00	21.20
9	Thilak	1006.67^{ab}	1466.67	29.04
10	TMV7	876.67^{ab}	1366.33	29.70
11	TMV6	933.33^{ab}	1566.67	39.23
12	TMV5	766.67^{abc}	1066.67	27.63
13	TMV4	686.67^{bcd}	1000.00	36.00
14	TMV3	973.33^{ab}	1344.00	27.70
15	*S. malabaricum*	980.00^{ab}	1416.67	24.33
	CD (0.05)	**321.49**	**NS**	

t-stat- 5.02 **t table (0.05)- 2.048**

Table 27. NRase activity of sesame genotypes waterlogged at flowering stage

Sl. No	Genotypes	NRase activity (µ moles of NO_2^- formed $g^{-1}hr^{-1}$)		Percentage reduction over control
		Waterlogged	Control	
1	SVPR 1	451.50^{bcd}	572.50^{cd}	21.14
2	CO1	360.00^{cde}	865.83^{bcd}	58.42
3	GT10	411.25^{cd}	510.50^{cd}	19.44
4	AT231	300.00^{de}	460.00^{d}	34.78
5	Ayali	462.50^{abcd}	620.00^{cd}	25.40
6	Thilatara	330.00^{de}	530.00^{cd}	37.74
7	Thilarani	587.50^{ab}	890.00^{a}	33.99
8	Kayamkulam 1	623.33^{a}	893.25^{ab}	30.22
9	Thilak	520.00^{abc}	816.67^{abc}	36.33
10	TMV7	338.33^{de}	507.50^{cd}	33.33
11	TMV6	417.50^{cd}	600.00^{cd}	30.42
12	TMV5	390.50^{cde}	546.50^{d}	28.55
13	TMV4	235.00^{e}	510.50^{cd}	53.97
14	TMV3	413.75^{cd}	687.50^{cd}	39.82
15	*S. malabaricum*	395.00^{cde}	593.33^{d}	33.43
	CD (0.05)	**164.697**	**224.254**	

t- stat- 4.722 **t table (0.05)- 2.048**

Waterlogging significantly reduced the nitrate reductase enzyme activity as indicated by higher t statistics (4.722) (Table 27). Under waterlogged condition, Kayamkulam 1 (623.33 µ moles of $NO_2^-g^{-1}hr^{-1}$), Thilarani (587.50 µ moles of $NO_2^-g^{-1}hr^{-1}$), Thilak (520.00 µ moles of $NO_2^-g^{-1}hr^{-1}$) and Ayali (462.50 µ moles of $NO_2^-g^{-1}hr^{-1}$) recorded higher NRase activity, whereas, TMV4 (235.00 µ moles of $NO_2^-g^{-1}hr^{-1}$) recorded the lowest activity which was statistically on par with AT231 (300 µ moles of $NO_2^-g^{-1}hr^{-1}$), Thilatara (330.00 µ moles of $NO_2^-g^{-1}hr^{-1}$), TMV7 (338.33 µ moles of $NO_2^-g^{-1}hr^{-1}$), CO 1 (360.00 µ moles of $NO_2^-g^{-1}hr^{-1}$), TMV5 (390.50 µ moles of $NO_2^-g^{-1}hr^{-1}$) and *Sesamum malabaricum* (395.00 µ moles of $NO_2^-g^{-1}hr^{-1}$). Lowest per cent reduction was observed in GT10 (19.44 %) and highest in CO1 (58.42 %) (Table 27).

4.2.1.3. Photosynthetic parameters

4.2.1.3.1. Photosynthetic rate (µ mole CO_2 $m^{-2}s^{-1}$)

Photosynthetic rate at vegetative stage varied among genotypes as shown in Table 28. TMV6 (21.75 µ mole CO_2 $m^{-2}s^{-1}$), CO1 (21.25 µ mole CO_2 $m^{-2}s^{-1}$), SVPR1 (20.75 µ mole CO_2 $m^{-2}s^{-1}$), TMV3 (20.75 µ mole CO_2 $m^{-2}s^{-1}$) and TMV 4 (20.25 µ mole CO_2 $m^{-2}s^{-1}$) recorded statistically higher photosynthetic rate than others. Waterlogging significantly reduced the photosynthetic rate as evident by the higher t- statistics (19.041). Under waterlogged condition, highest photosynthetic rate was observed in Ayali (8.30 µ mole CO_2 $m^{-2}s^{-1}$) followed by SVPR1 (6.77 µ mole CO_2 $m^{-2}s^{-1}$), Thilarani (6.70 µ mole CO_2 $m^{-2}s^{-1}$), TMV5 (6.53 µ mole CO_2 $m^{-2}s^{-1}$) and TMV7 (6.30 µ mole CO_2 $m^{-2}s^{-1}$) whereas lowest was recorded from CO1 (1.70 µ mole CO_2 $m^{-2}s^{-1}$), AT231 (1.83 µ mole CO_2 $m^{-2}s^{-1}$), Thilatara (2.20 µ mole CO_2 $m^{-2}s^{-1}$) and TMV4 (2.40 µ mole CO_2 $m^{-2}s^{-1}$).

Photosynthetic rate at flowering period ranged from 16.67 µ mole CO_2 $m^{-2}s^{-1}$ in TMV4 to 23.75 µ mole CO_2 $m^{-2}s^{-1}$ in TMV6 (Table 29). Waterlogging reduced the photosynthetic rate of all genotypes. The genotypes CO1 (7.33 µ mole CO_2 $m^{-2}s^{-1}$), AT231 (8.67 µ mole CO_2 $m^{-2}s^{-1}$) and TMV4 (8.83 µ mole CO_2 $m^{-2}s^{-1}$) recorded lowest photosynthetic rate under waterlogged condition, while all others recorded the highest.

Table 28. Photosynthetic rate of sesame genotypes waterlogged at vegetative stage

Sl. No	Genotypes	Photosynthetic rate (µ mole CO_2 $m^{-2}s^{-1}$)	
		Waterlogged	Control
1	SVPR 1	6.77^{b}	20.75^{ab}
2	CO1	1.70^{g}	21.25^{ab}
3	GT10	4.77^{ef}	17.75^{bcd}
4	AT231	1.83^{g}	16.25^{d}
5	Ayali	8.30^{a}	18.00^{bcd}
6	Thilatara	2.20^{g}	17.75^{bcd}
7	Thilarani	6.70^{bc}	17.00^{cd}
8	Kayamkulam 1	5.50^{de}	17.00^{cd}
9	Thilak	3.83^{f}	17.00^{cd}
10	TMV7	6.30^{bcd}	17.00^{cd}
11	TMV6	5.17^{e}	21.75^{a}
12	TMV5	6.53^{bc}	17.00^{cd}
13	TMV4	2.40^{g}	20.25^{abc}
14	TMV3	5.73^{cde}	20.75^{ab}
15	*S. malabaricum*	5.43^{de}	18.00^{bcd}
	CD (0.05)	1.014	3.652

t stat- 19.041 t table (0.05)- 2.048

Table 29. Photosynthetic rate of sesame genotypes waterlogged at flowering stage

Sl. No	Genotypes	Photosynthetic rate (µ mole CO_2 $m^{-2}s^{-1}$)	
		Waterlogged	Control
1	SVPR 1	13.33^{a}	21.33^{ab}
2	CO 1	7.33^{d}	22.00^{a}
3	GT 10	10.67^{abc}	17.33^{cd}
4	AT 231	8.67^{cd}	16.67^{d}
5	Ayali	12.33^{a}	20.00^{abcd}
6	Thilatara	10.40^{abcd}	22.00^{a}
7	Thilarani	11.87^{ab}	21.33^{ab}
8	Kayamkulam 1	10.33^{abcd}	16.67^{d}
9	Thilak	10.67^{abc}	18.00^{bcd}
10	TMV 7	12.67^{a}	23.33^{a}
11	TMV 6	10.33^{abcd}	23.67^{a}
12	TMV 5	11.20^{abc}	21.67^{ab}
13	TMV 4	8.83^{bcd}	16.67^{d}
14	TMV 3	12.00^{a}	20.67^{abc}
15	*Sesamum malabaricum*	11.67^{abc}	20.33^{abcd}
	CD	3.134	3.984

t-statistics- 12.267 t table (0.05)- 2.048

4.2.1.3.2. Transpiration rate (µmol H_2O $m^{-2}s^{-1}$)

Transpiration rate was varied among genotypes as indicated in Table 30. TMV3 (9.97 µmol H_2O $m^{-2}s^{-1}$), TMV6 (9.95 µmol H_2O $m^{-2}s^{-1}$), TMV4 (9.87 µmol H_2O $m^{-2}s^{-1}$), CO1 (9.77 µmol H_2O $m^{-2}s^{-1}$), SVPR1 (99.75 µmol H_2O $m^{-2}s^{-1}$), Ayali (9.50 µmol H_2O $m^{-2}s^{-1}$) and Thilatara (8.98 µmol H_2O $m^{-2}s^{-1}$) recorded higher transpiration. Waterlogging resulted in decline in transpiration rate as indicated by higher t-statistics (18.774). Under waterlogged condition, Thilarani (5.40 µmol H_2O $m^{-2}s^{-1}$) and Ayali (5.40 µmol H_2O $m^{-2}s^{-1}$) recorded highest transpiration rate whereas CO1 (1.75 µmol H_2O $m^{-2}s^{-1}$) and AT231 (1.98 µmol H_2O $m^{-2}s^{-1}$) recorded the lowest.

At flowering stage in control condition, transpiration rate was found to be non-significant (Table 31). Waterlogging significantly reduced the transpiration rate as evident by higher t-statistics (17.252). Significant variation under waterlogged condition was observed. Eight genotypes recorded higher values (TMV7, Thilarani, TMV3, Ayai, Kayamkulam 1, SVPR1, GT 10 and *Sesamum malabaricum*). CO 1 (3.95 µmol H_2O $m^{-2}s^{-1}$), TMV4 (4.27 µmol H_2O $m^{-2}s^{-1}$), TMV5 (4.67 µmol H_2O $m^{-2}s^{-1}$) and Thilatara (4.73 µmol H_2O $m^{-2}s^{-1}$) recorded lower values.

4.2.1.3.3. Stomatal conductance (µmol $m^{-2}s^{-1}$)

Variation in stomatal conductance at vegetative stage are given in Table 32. In control condition, TMV6 (0.545 µmol $m^{-2}s^{-1}$), CO1 (0.540 µmol $m^{-2}s^{-1}$) and TMV 3 (0.530 µmol $m^{-2}s^{-1}$) recorded highest stomatal conductance. Waterlogging reduced the stomatal conductance as evident by higher t-statistics (13.704). Under waterlogged condition, Thilarani (0.216 µmol $m^{-2}s^{-1}$), Ayali (0.205 µmol $m^{-2}s^{-1}$), TMV5 (0.196 µmol $m^{-2}s^{-1}$) and *S. malabaricum* (0.173 µmol $m^{-2}s^{-1}$) recorded the highest stomatal conductance. The genotype AT231 (0.060 µmol $m^{-2}s^{-1}$) recorded the lowest which was statistically on par with Thilatara (0.083 µmol $m^{-2}s^{-1}$), TMV4 (0.081 µmol $m^{-2}s^{-1}$), CO1 (0.085 µmol $m^{-2}s^{-1}$), Kayamkulam 1 (0.109 µmol $m^{-2}s^{-1}$) and TMV 3 (0.115 µmol $m^{-2}s^{-1}$).

Stomatal conductance varied among genotypes at flowering stage (Table 33). TMV4 (0.580 µmol $m^{-2}s^{-1}$), Kayamkulam 1 (0.590 µmol $m^{-2}s^{-1}$), AT231 (0.633 µmol

Table 30. Transpiration rate of sesame genotypes waterlogged at vegetative stage

Sl. No	Genotypes	Transpiration rate (µmol H_2O $m^{-2}s^{-1}$)	
		Waterlogged	Control
1	SVPR 1	3.23^{de}	9.75^{abcd}
2	CO 1	1.75^{i}	9.77^{abcd}
3	GT 10	2.73^{gh}	8.78^{de}
4	AT 231	1.98^{i}	8.10^{e}
5	Ayali	5.40^{a}	9.50^{abcd}
6	Thilatara	2.90^{fg}	8.98^{abcde}
7	Thilarani	5.40^{a}	8.43^{e}
8	Kayamkulam 1	4.23^{b}	8.90^{cde}
9	Thilak	3.23^{de}	8.73^{de}
10	TMV 7	3.33^{de}	8.80^{de}
11	TMV 6	2.50^{h}	9.95^{ab}
12	TMV 5	3.64^{c}	8.91^{bcde}
13	TMV 4	3.45^{cd}	9.87^{abc}
14	TMV 3	3.27^{de}	9.97^{a}
15	*S. malabaricum*	3.08^{ef}	8.85^{cde}
	CD	0.288	1.043

t-stat- 18.774 **t table (0.05)- 2.048**

Table 31. Transpiration rate of sesame genotypes waterlogged at flowering stage

Sl. No	Genotypes	Transpiration rate (µmol H_2O $m^{-2}s^{-1}$)	
		Waterlogged	Control
1	SVPR 1	6.07^{ab}	10.08
2	CO 1	3.95^{f}	10.17
3	GT 10	6.00^{ab}	9.50
4	AT 231	5.43^{bcd}	8.77
5	Ayali	6.17^{ab}	9.50
6	Thilatara	4.73^{cdef}	10.27
7	Thilarani	6.50^{a}	10.50
8	Kayamkulam 1	6.17^{ab}	9.37
9	Thilak	5.50^{bc}	10.07
10	TMV 7	6.57^{a}	10.07
11	TMV 6	5.03^{cde}	9.62
12	TMV 5	4.67^{def}	9.67
13	TMV 4	4.27^{ef}	9.47
14	TMV 3	6.17^{ab}	10.00
15	*S.malabaricum*	5.93^{ab}	9.33
	CD	0.802	NS

t stat- 17.252 **t table (0.05)- 2.048**

Table 32. Stomatal conductance of sesame genotypes waterlogged at vegetative stage

Sl. No	Genotypes	Stomatal conductance (µmol $m^{-2}s^{-1}$)	
		Waterlogged	Control
1	SVPR 1	0.152^{bcd}	0.473^{cd}
2	CO 1	0.085^{ef}	0.540^{ab}
3	GT 10	0.121^{cde}	0.398^{ef}
4	AT 231	0.060^{f}	0.368^{f}
5	Ayali	0.205^{ab}	0.373^{f}
6	Thilatara	0.083^{ef}	0.453^{de}
7	Thilarani	0.216^{a}	0.380^{f}
8	Kayamkulam 1	0.109^{def}	0.363^{f}
9	Thilak	0.137^{cde}	0.360^{f}
10	TMV 7	0.125^{cde}	0.360^{f}
11	TMV 6	0.124^{cde}	0.545^{a}
12	TMV 5	0.196^{ab}	0.390^{f}
13	TMV 4	0.081^{ef}	0.480^{bcd}
14	TMV 3	0.115^{def}	0.530^{abc}
15	*S. malabaricum*	0.173^{abc}	0.468^{d}
	CD	0.057	0.062

t stat- 13.704 **t table (0.05)- 2.048**

Table 33. Stomatal conductance of sesame genotypes waterlogged at flowering stage

Sl. No	Genotypes	Stomatal conductance (µmol $m^{-2}s^{-1}$)	
		Waterlogged	Control
1	SVPR 1	0.335^{c}	0.677^{ab}
2	CO 1	0.197^{d}	0.690^{ab}
3	GT 10	0.347^{c}	0.633^{bc}
4	AT 231	0.350^{c}	0.633^{bc}
5	Ayali	0.410^{c}	0.660^{abc}
6	Thilatara	0.247^{d}	0.697^{ab}
7	Thilarani	0.450^{a}	0.727^{a}
8	Kayamkulam 1	0.410^{c}	0.590^{c}
9	Thilak	0.380^{bc}	0.650^{abc}
10	TMV 7	0.430^{ab}	0.711^{ab}
11	TMV 6	0.333^{c}	0.663^{abc}
12	TMV 5	0.447^{a}	0.683^{ab}
13	TMV 4	0.347^{c}	0.580^{c}
14	TMV 3	0.437^{ab}	0.638^{bc}
15	*S. malabaricum*	0.427^{ab}	0.663^{abc}
	CD	0.059	0.084

t stat- 13.309 **t table (0.05)- 2.048**

$m^{-2}s^{-1}$), GT10 (0.633 µmol $m^{-2}s^{-1}$) and TMV3 (0.638 µmol $m^{-2}s^{-1}$) recorded lower stomatal conductance under control condition compared to others. Waterlogging significantly reduced the stomatal conductance as evident in higher t-statistics (13.309). At waterlogged condition, CO1 (0.197 µmol $m^{-2}s^{-1}$) and Thilaara (0.247 µmol $m^{-2}s^{-1}$) recorded lowest stomatal conductance, whereas, Thilarani (0.450 µmol $m^{-2}s^{-1}$), TMV5 (0.447 µmol $m^{-2}s^{-1}$), TMV3 (0.437 µmol $m^{-2}s^{-1}$), TMV7 (0.430 µmol $m^{-2}s^{-1}$), *Sesamum malabaricum* (0.427 µmol $m^{-2}s^{-1}$), Kayamkulam 1 (0.410 µmol $m^{-2}s^{-1}$) and Ayali (0.410 µmol $m^{-2}s^{-1}$) recorded the highest (Table 33).

4.2.4. Anaerobic enzyme activity in roots

The activities of LDH, ADH and PDC were recorded from TMV 4, CO1, Thilatara and AT231 which recorded lower survival and from *S. malabaricum*, Thilarani, Ayali and SVPR1 recorded higher survival at vegetative stage waterlogging. The enzyme activity was estimated from roots of waterlogged (vegetative stage) and control plants.

4.2.4.1. Lactate dehydrogenase (LDH) activity (unit g^{-1} protein)

Significant difference was observed in LDH activity (Table 34). Waterlogging increased the LDH activity of 3 genotypes viz., CO 1, Thilatara and AT231. In other five genotypes, it was found to be decreasing. Under waterlogged condition, AT231 (3.57 unit g^{-1} protein) recorded highest LDH activity and SVPR1 (0.27 unit g^{-1} protein) recorded the lowest.

4.2.4.2. Alcohol dehydrogenase (ADH) activity (unit g^{-1} protein)

Significant difference was observed in ADH activity as seen in Table 35. Thilarani (9.06 unit g^{-1} protein) and TMV4 (8.49 unit g^{-1} protein) recorded highest ADH activity under waterlogged condition, whereas, CO 1 (0.22 unit g^{-1} protein) recorded the lowest. Higher increase in ADH activity under waterlogged condition compared to control was observed in genotypes Ayali, Thilarani, SVPR1 and *S. malabaricum*.

Table 34. LDH activity (unit g^{-1} protein) of sesame genotypes waterlogged at vegetative stage

Sl.no	Genotypes	Waterlogged	Control	Percentage change over control
1	TMV4	1.88[c]	3.43[b]	-45.14
2	CO 1	1.92[c]	0.25[f]	668.00
3	Thilatara	2.66[b]	1.77[c]	50.09
4	AT 231	3.57[a]	0.97[d]	269.31
5	Ayali	0.89[d]	3.94[a]	-77.39
6	Thilarani	0.49[e]	0.71[e]	-30.99
7	SVPR 1	0.27[f]	1.90[c]	-85.94
8	*Sesamum malabaricum*	0.59[e]	1.01[d]	-42.22
	CD (0.05)	0.179	0.129	

Table 35. ADH activity (unit g^{-1} protein) of sesame genotypes waterlogged at vegetative stage

Sl.no	Genotypes	Waterlogged	Control	Percentage change over control
1	TMV4	8.49[ab]	7.24[b]	17.37
2	CO 1	0.22[g]	1.32[ef]	-83.04
3	Thilatara	4.64[d]	8.49[a]	-45.37
4	AT 231	2.20[f]	1.81[e]	21.77
5	Ayali	3.65[e]	0.98[f]	272.45
6	Thilarani	9.06[a]	3.24[c]	179.73
7	SVPR 1	8.13[b]	3.36[c]	142.20
8	*Sesamum malabaricum*	5.40[c]	2.53[d]	113.16
	CD (0.05)	0.687	0.620	

Table 36. PDC (unitg^{-1}protein) activity of sesame genotypes waterlogged at vegetative stage

Sl.no	Genotypes	Waterlogged	Control	Percentage change over control
1	TMV4	3.85[c]	3.13[bc]	23.20
2	CO 1	3.63[c]	3.38[bc]	7.41
3	Thilatara	4.97[c]	4.08[b]	21.84
4	AT 231	3.53[c]	2.45[c]	43.88
5	Ayali	28.50[a]	7.25[a]	293.10
6	Thilarani	26.50[a]	8.00[a]	231.25
7	SVPR 1	17.50[b]	4.45[b]	293.26
8	*Sesamum malabaricum*	29.00[a]	8.00[a]	262.50
	CD (0.05)	5.158	1.504	

4.2.4.3. Pyruvate decarboxylase (PDC) activity (unit g^{-1} protein)

Significant difference was observed in PDC activity (Table 36). *S. malabaricum* (29.00 unit g^{-1} protein), Ayali (28.50 unit g^{-1} protein) and Thilarani (28.50 unit g^{-1} protein) recorded highest PDC activity under waterlogged condition. TMV4 (3.53 unit g^{-1} protein), CO1 (3.63 unit g^{-1} protein), Thialtara (4.97 unit g^{-1} protein) and AT231 (3.53 unit g^{-1} protein) recorded the lowest. CO1 recorded lowest percent increase whereas, Ayali and SVPR recorded highest per cent increase in PDC activity.

4.2.1.5 Anatomical study

Root cross sections taken at the end of waterlogging was examined under microscope from waterlogged and non-waterlogged plants of *S.malabaricum*, Kayamulam1, Ayali and CO1 (Plate 7-10). Formation of aerenchyma was observed in the genotypes *S.malabaricum*, Kayamulam1 and Ayali. In the genotype CO 1 no significant formation of aerenchyma was observed.

Plate 7A. Kayamkulam 1 (control)

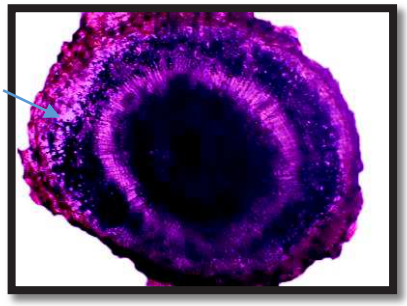


Plate 7B. Waterlogged

Plate 7. Root cross section of sesame var. Kayamkulam 1 in control **(A)** and after waterlogging **(B)**. Blue arrow indicates aerenchyma formation

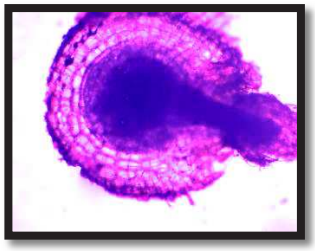


Plate 8A. Control

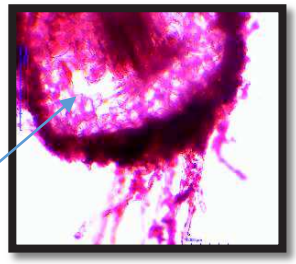
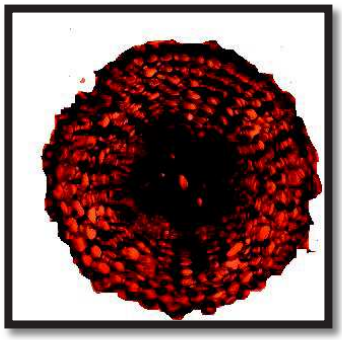


Plate 8 B. Waterlogged

Plate 8. Root cross section of sesame var. Ayali in control **(A)** after waterlogging **(B)**. Blue arrow indicates aerenchyma formation



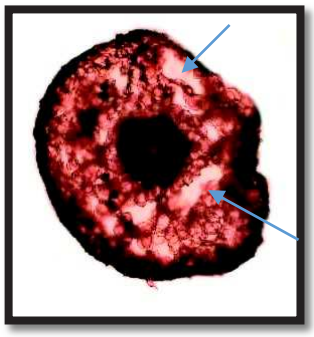
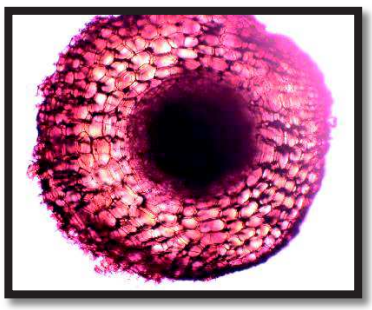


Plate 9A. Control **Plate 9B. Waterlogged**

Plate 9 Root cross section of *S. malabaricum* in control **(A)** and after waterlogging **(B)**. Blue arrow indicated aerenchyma formation.



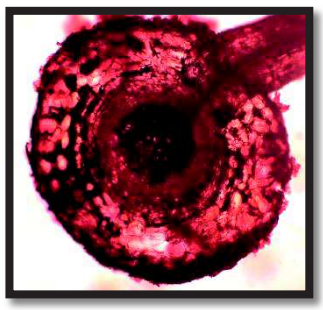


Plate 10 A. Control **Plate 10B. Waterlogged**

Plate 10. Root cross section of CO 1 in control **(A)** and after waterlogging **(B)**

4.2.2 SCREENING FOR DROUGHT STRESS TOLERANCE IN SESAME

A lab study was conducted to screen 15 sesame varieties for tolerance to drought at seedling stage. Screening was done using aqueous solution of PEG 6000. Percentage of normal seedlings, abnormal seedlings, dead seeds, hard seeds and fresh and ungerminated seeds, shoot length, root length, root to shoot ratio, speed of germination, vigour index and stress tolerance index of all the varieties were calculated. The details of the study are given below.

4.2.2.1 Germination percentage (%)

Evaluation of seed germination was carried out by taking the percentage of normal, abnormal, dead, hard, fresh and ungerminated seeds in control and PEG treated seeds (Table 37, 38).

Percentage of normal seedlings (Table 37) were lower in PEG treatment compared to control as evident by the higher t statistics (7.599) compared to table t value (2.048). The varieties which showed higher per cent of normal seedlings under drought stress were Kayamkulam 1 (66.67 %), TMV3 (66.08 %), Ayali (65.00), Thilatara (62.22 %) and TMV6 (56.23%). The varieties recorded lowest per cent of normal seedlings were AT231 (28.33 %), CO1 (28.89 %), GT10 (31.11%), *Sesame malabaricum* (35.55%) and TMV 5 (38.33%). Under control condition, Thilatara (96.67%), SVPR1 (96.67%), CO1 (95.56%), TMV4 (95.56%), TMV5 (95.56%), TMV7 (95.00%), Kayalkulam 1 (95.00%), Ayali (95.00%), and TMV3 (93.33%) recorded higher per cent of normal seedlings, whereas AT231 (56.67%) and *Sesamum malabaricum* (55.56%) recorded lowest germination.

The percentage of abnormal seedlings were taken in both control and stress condition (Table 37). It was observed that number of abnormal seedling increased under drought stress as indicated by higher t statistics (10.977). Among the genotypes, AT231 (8.00 %), Thilak (7.78 %) and TMV6 (6.67%) recorded comparitively higher per cent of abnormal seedlings under control condition. Under drought stress condition, CO1 (52.78 %), TMV 7 (50.00%), TMV5 (48.25 %),

Table 37. Percentage of normal seedlings, abnormal seedlings and dead seeds in sesame genotypes under PEG induced drought stress

Sl. no	Genotypes	Normal seedlings (%)		Abnormal seedlings (%)		Dead seeds (%)	
		Control	PEG	Control	PEG	Control	PEG
1	Thilak	88.89^{c} (1.234)	51.66^{cd} (0.802)	7.78^{a} (2.782)	28.33^{de} (0.559)	0.00^{f} (0.707)	12.92^{bcd} (3.553)
2	SVPR1	96.67^{a} (1.440)	53.33^{cd} (0.819)	3.33de (1.823)	31.94^{cde} (0.587)	0.00^{f} (0.707)	10.00^{cdef} (3.117)
3	*S. malabaricum* (wild sesame)	55.56^{e} (0.841)	35.55^{ef} (0.634)	5.50^{bc} (2.343)	28.33^{de} (0.554)	4.45^{d} (2.223)	2.00^{g} (1.414)
4	AT 231	56.67^{e} (0.853)	28.33^{f} (0.559)	8.00^{a} (2.821)	43.89^{ab} (0.724)	17.84^{a} (4.277)	21.67^{a} (4.645)
5	Thilarani	90.00^{bc} (1.259)	54.25^{bc} (0.828)	4.33^{cde} (1.998)	26.67^{de} (0.543)	5.67^{c} (2.467)	12.41^{bcd} (3.523)
6	Thilatara	96.67^{a} (1.440)	62.22^{abc} (0.909)	3.33^{de} (1.823)	25.00^{de} (0.523)	0.00^{f} (0.707)	12.78^{bcd} (3.559)
7	Kayamkulam 1	95.00^{ab} (1.412)	66.67^{a} (0.956)	1.67^{f} (1.286)	22.78^{e} (0.490)	1.67^{e} (1.467)	10.56^{cdef} (3.19)
8	GT10	73.33^{d} (1.028)	31.11^{f} (0.584)	3.33^{de} (1.823)	41.11^{abc} (0.696)	13.33^{b} (3.71)	17.78^{ab} (4.2)
9	Ayali	95.00^{abc} (1.375)	65.00^{ab} (0.939)	1.50^{f} (1.213)	20.56^{e} (0.469)	2.00^{e} (1.577)	7.78^{ef} (2.768)
10	CO1	95.56^{abc} (1.387)	28.89^{f} (0.563)	2.78^{e} (1.664)	52.78^{a} (0.813)	1.67^{e} (1.469)	18.33^{ab} (4.12)
11	TMV3	93.33^{abc} (1.310)	66.08^{a} (0.950)	3.33^{de} (1.823)	26.75^{de} (0.543)	0.00^{f} (0.707)	7.17^{f} (2.677)
12	TMV4	95.56^{abc} (1.387)	53.73^{c} (0.823)	4.44^{cd} (2.066)	34.08^{bcd} (0.623)	0.00^{f} (0.707)	12.19^{bcde} (3.476)
13	TMV5	95.56^{abc} (1.387)	38.33^{ef} (0.667)	4.45^{cd} (2.106)	48.25^{a} (0.768)	0.00^{f} (0.707)	13.42^{bc} (3.657)
14	TMV6	88.89^{c} (1.234)	56.23^{abc} (0.848)	6.67^{ab} (2.583)	34.33^{bcd} (0.626)	4.44^{d} (2.214)	9.44^{cdef} (3.058)
15	TMV7	95.00^{abc} (1.375)	42.00^{de} (0.705)	3.33^{de} (1.823)	50.00^{a} (0.785)	1.67^{e} (1.469)	8.00^{def} (2.821)
	Mean	87.45	48.89	4.251	34.32	3.52	11.76
	CD (0.05)	**0.157**	**0.116**	**0.348**	**0.118**	**0.230**	**0.743**
	t statistics	**7.599**		**10.977**		**4.411**	

t table (0.05)- 2.048, Values in parenthesis indicate arc sin transformation

Table 38. Percentage of hard seeds and fresh and ungerminated seeds in sesame genotypes under PEG induced drought stress

Sl. no	Genotypes	Hard seeds (%)		Fresh and ungerminated seeds (%)	
		Control	PEG	Control	PEG
1	Thilak	3.34	0.00	0.00 (0.707)	7.09 ^{c} (2.752)
2	SVPR1	0.00	0.00	0.00 (0.707)	4.73^{d} (2.279)
3	*S.malabaricum* (wild sesame)	17.50	21.35	17.00 (4.114)	12.77^{a} (3.597)
4	AT 231	0.00	0.00	17.50 (4.238)	6.11^{cd} (2.564)
5	Thilarani	0.00	0.00	0.00 (0.707)	6.67^{c} (2.678)
6	Thilatara	0.00	0.00	0.00 (0.707)	0.00^{e} (0.707)
7	Kayamkulam 1	0.00	0.00	1.67 (1.467)	0.00^{e} (0.707)
8	GT10	0.00	0.00	10.01 (3.233)	10.00 ^{b} (3.198)
9	Ayali	0.00	0.00	1.50 (1.168)	6.67^{c} (2.678)
10	CO1	0.00	0.00	0.00 (0.707)	0.00^{e} (0.707)
11	TMV3	0.00	0.00	3.34 (1.951)	0.00^{e} (0.707)
12	TMV4	0.00	0.00	0.00 (0.707)	0.00^{e} (0.707)
13	TMV5	0.00	0.00	0.00 (0.707)	0.00^{e} (0.707)
14	TMV6	0.00	0.00	0.00 (0.707)	0.00^{e} (0.707)
15	TMV7	0.00	0.00	0.00 (0.707)	0.00 ^{e} (0.707)
	CD (0.05)	**3.361**		**0.494**	**0.351**
	t statistics	**0.018**		**0.103**	

t table (0.05)- 2.048, Values in parenthesis indicate square root transformation

AT231 (43.89 %) and GT10 (41.11 %) recorded higher per cent of abnormal seedlings whereas other genotypes recorded comparatively lower abnormal seedlings.

The percentage of dead seeds (Table 37) were increased under PEG treatment as indicted by higher t statistics (4.411) value compared to table t value (2.048). Under control condition, the genotypes AT231 (17.84 %) followed by GT 10 (13.33%) recorded highest number of dead seeds whereas the genotypes such as TMV4, Thilatara, Thilak, TMV3, TMV5 and SVPR1 recorded zero percent of abnormal seedlings. Under stress condition, AT 231 (21.67%), CO 1 (18.33 %) and GT10 (17.78%) recorded higher per cent of dead seeds whereas *Sesamum malabaricum* (2.00 %) recorded the lowest.

Under control condition, hard seeds (Table 38) were recorded only from *Sesamum malabaricum* and Thilak (3.34 %) where the former recorded the highest per cent of 17.50 per cent (Table 38). Under drought stress condition, hard seeds were recorded from *Sesamum malabaricum* only with 21.35 per cent. The percentage of hard seeds (Table 38) were found to be increased under PEG induced drought stress in *S. malabaricum*. The percentage of fresh and ungerminated seeds (Table 38) were found to be not affected with PEG treatment as the lower t-statistics indicate (0.103).Under control condition, fresh and ungerminated seedlings were observed in 6 genotypes such as, AT231 (17.50 %, *Sesamum malabaricum* (17.00%), GT10 (10.01 %), TMV3 (3.34 %), Kayamkulam 1 (1.67%) and Ayali (1.50 %). Under stress condition, *Sesamum malabaricum* (12.77%), GT10 (10.00%), Thilak (7.09%), Thilarani (6.67 %), Ayali (6.67 %), AT231 (6.11%) and SVPR1 (4.73%) recorded fresh and ungerminated seeds whereas others showed zero per cent.

4.2.2.2 Shoot length (cm)

Variation in shoot length of sesame seedlings under PEG induced drought stress is given in Table 39. Shoot length was decreased under PEG treatment in all sesame varieties indicated by the higher t statistics (7.941) compared to table t value. In drought stress condition, TMV3 (3.05 cm) followed by AT231 (2.70 cm) recorded highest shoot length. In control condition, CO 1 (6.65 cm) and TMV 7 (6.37 cm)

recorded highest shoot length. In both conditions wild sesame recorded the lowest shoot length.

Variation in percentage reduction in shoot length over control in each genotype was estimated (Table 39). *S.malabaricum* recorded the least reduction in shoot length (30.59%) whereas CO1 recorded the highest reduction (71.39%).

4.2.2.3 Root length (cm)

The change in root length upon induced drought stress in sesame varieties are given in Table 40. Higher t statistics (12.63) compared to table t value indicate reduction in root length under drought stress (Table 40).

In stress condition TMV3 (2.13 cm), Ayali (2.10 cm), Kayamkulam 1(2.00cm) and Thilatara (1.93 cm) showed highest root length. The genotypes AT 231(1.07 cm), *Sesamum malabaricum* (1.08 cm), Thilak (1.10cm), TMV 7 (1.17 cm), GT 10 (1.24 cm) and CO 1 (1.25 cm) recorded the lowest. In control condition, highest root length was observed in Thilak (7.33 cm), TMV 5 (7.15 cm) and TMV6 (6.79 cm) and CO 1(6.73 cm). Lowest root length was observed in S*esamum malabaricum* (2.49 cm) (Table 40).

Variation in per cent reduction in root length was observed among genotypes (table 40). Thilak (85.00 %) recorded highest whereas *S.malabaricum* (56.76 %) recorded the least per cent reduction in root length (Table 40).

Table 39. Response of shoot length (cm) to PEG treatment in sesame varieties

Sl. no	Genotypes	Shoot length (cm)		Percentage reduction over control
		Control	PEG	
1	Thilak	5.57^{c}	2.16^{cd}	61.31
2	SVPR1	4.40^{de}	2.53^{bc}	42.47
3	*S.malabaricum*	1.84^{f}	1.28^{e}	30.59
4	AT 231	4.65^{d}	2.70^{ab}	41.94
5	Thilarani	4.05^{e}	2.34^{bcd}	42.35
6	Thilatara	4.67^{d}	2.50^{bc}	46.50
7	Kayamkulam 1	4.40^{de}	2.60^{abc}	40.91
8	GT10	4.48^{de}	2.45^{bc}	45.40
9	Ayali	4.33^{de}	2.57^{bc}	40.77
10	CO1	6.65^{a}	1.90^{d}	71.39
11	TMV3	5.90^{bc}	3.05^{a}	48.33
12	TMV4	5.57^{c}	2.52^{bc}	54.70
13	TMV5	6.02^{bc}	2.18^{cd}	63.84
14	TMV6	5.94^{bc}	2.37^{bc}	60.04
15	TMV7	6.37^{ab}	2.47^{bc}	61.22
	Mean	4.989	2.375	
	CD (0.05)	0.520	0.470	

t statistics : 7.941 **t table (0.05):2.048**

Table 40. Response of root length (cm) in sesame varieties to PEG treatment

Sl. no	Genotypes	Root length (cm)		Percentage reduction over control
		PEG	Control	
1	Thilak	1.10^{g}	7.33^{a}	85.00
2	SVPR1	1.50^{def}	5.23^{efg}	71.29
3	*Sesamum malabaricum*	1.08^{g}	2.49^{h}	56.76
4	AT 231	1.07^{g}	4.67^{g}	77.14
5	Thilarani	1.70^{cd}	5.63^{def}	69.78
6	Thilatara	1.93^{abc}	6.05^{cd}	68.04
7	Kayamkulam 1	2.00^{abc}	6.33^{bc}	68.42
8	GT10	1.24^{fg}	5.50^{def}	77.50
9	Ayali	2.10^{ab}	5.88^{cde}	64.26
10	CO1	1.25^{efg}	6.73^{ab}	81.38
11	TMV3	2.13^{a}	5.57^{def}	61.68
12	TMV4	1.79^{bcd}	4.67^{g}	61.57
13	TMV5	1.52^{def}	7.15^{a}	78.79
14	TMV6	1.56^{de}	6.79^{ab}	77.10
15	TMV7	1.17^{g}	5.21f^{g}	77.49
	Mean	1.543	5.682	
	CD (0.05)	0.313	0.657	

t statistics : 12.63 **t table (0.05)- 2.048**

4.2.2.4 Speed of germination

The speed of germination of sesame varieties under control and stress conditions were given in Table 41. Speed of germination was not affected with PEG induced stress as the lower t-statistics (0.604) indicate. In both conditions, *S. malabaricum* and AT 231 recorded lowest speed of germination.

4.2.2.5 Vigour index

The vigour index of sesame varieties under control and drought stress conditions are given in Table 42. Drought stress reduced the vigour index of sesame varieties as indicated by higher t statistics (10.149). In control, CO 1 (1278) and TMV 5 (1259) recorded highest vigour index while, *Sesamum malabaricum* (240) recorded the lowest. In stress condition, TMV 3 (342), Kayamkulam 1 (308) and Ayali (304) recorded the highest while *Sesamum malabaricum* (85), CO1 (91), AT231 (106) and GT10 (117) recorded the lowest.

S. malabaricum (64.68 %) recorded lowest percentage reduction in seedling vigour over control, whereas CO1 (92.86%) recorded highest reduction (Table 42).

4.2.2.6 Stress tolerance index

Among the genotypes under investigation, higher stress tolerance index was recorded in *S.malabaricum* (35), TMV3 (32), Ayali (32), Kayamkulam 1 (31) and Thilatara (27). The varieties CO1 (7), TMV5 (11), TMV7 (14) and Thilak (15) recorded the lowest stress tolerance index (Table 43).

Table 41. Speed of germination of sesame varieties in response to PEG treatment

Sl. no	Genotypes	Speed of germination	
		Control	PEG
1	Thilak	98^{ab}	93^{abcd}
2	SVPR1	97^{abc}	90^{bcd}
3	*S. malabaricum* (Wild sesame)	27^{f}	24^{h}
4	AT 231	35^{e}	32^{g}
5	Thilarani	98^{abc}	90^{cd}
6	Thilatara	97^{abc}	93^{abc}
7	Kayamkulam 1	96^{abc}	92^{bcd}
8	GT10	85^{d}	79^{f}
9	Ayali	91^{bcd}	87^{de}
10	CO1	90cd	82^{ef}
11	TMV3	96^{abc}	91^{bcd}
12	TMV4	100^{a}	96^{ab}
13	TMV5	100^{a}	91^{bcd}
14	TMV6	100^{a}	98^{a}
15	TMV7	100^{a}	96^{ab}
	Mean	87	82
	CD (0.05)	7.838	5.561

t statistics: 0.604 Table t value (0.05): 2.048

Table 42. Vigour index of sesame varieties in response to PEG treatment

Sl. no	Genotypes	Vigour index		Percentage reduction over control
		PEG	Control	
1	Thilak	168^{ef}	1147^{b}	85.32
2	SVPR1	218^{de}	930^{ef}	76.58
3	*S.malabaricum*	85^{i}	240^{i}	64.68
4	AT 231	106^{ghi}	529^{h}	79.90
5	Thilarani	219^{de}	870^{f}	74.87
6	Thilatara	276^{bc}	1036^{cd}	73.37
7	Kayamkulam 1	308^{ab}	1021^{cd}	69.88
8	GT10	117^{fghi}	732^{g}	83.99
9	Ayali	304^{ab}	966^{de}	68.52
10	CO1	91^{hi}	1278^{a}	92.86
11	TMV3	342^{a}	1070^{bc}	68.05
12	TMV4	232^{cd}	977^{de}	76.29
13	TMV5	142^{fgh}	1258^{a}	88.75
14	TMV6	221^{cde}	1129^{b}	80.41
15	TMV7	153^{fg}	1100^{bc}	86.09
	Mean	198.80	952.20	
	CD (0.05)	54.897	90.118	

t statistics : 10.149 t table (0.05)- 2.048

Table 43. Stress tolerance index of sesame genotypes

Sl. No	Genotypes	Stress tolerance index
1	Thilak	14.71^{efg}
2	SVPR1	23.58^{bcd}
3	*Sesamum malabaricum*	35.15^{a}
4	AT 231	19.98^{cde}
5	Thilarani	25.27^{bc}
6	Thilatara	26.74^{abc}
7	Kayamkulam 1	30.71^{ab}
8	GT10	16.23^{def}
9	Ayali	31.84^{ab}
10	CO1	7.17^{g}
11	TMV3	32.01^{ab}
12	TMV4	23.74^{bcd}
13	TMV5	11.28^{fg}
14	TMV6	19.73^{cdef}
15	TMV7	13.90^{efg}
	CD	8.618

4.3 MOLECULAR CHARACTERIZATION OF SESAME GENOTYPES

Results of the molecular characterization of sesame genotypes using candidate genes and markers reported for waterlogging tolerance are discussed hereunder.

4.3.1 Isolation and quantification of total genomic DNA

A good concentration of total DNA was isolated from the leaves of sesame genotypes using CTAB method (Table 44). The DNA was quantified using NanoDrop® spectrophotometer ND 1000. 1 ul of DNA sample was loaded and the absorbance were measured at 260 and 280 nm. All the DNA samples had good quality with $A_{260/280}$ values at 1.8-2.0. Gel documentation of isolated DNA was done to check the integrity of isolated DNA samples (Plate 11).

Table 44. The concentrations of the DNA samples obtained

Genotypes	**Quantity** (µg/ µl)
Sesamum malabaricum	0.555
SVPR 1	0.768
Kayankulam 1	1.5
Thilarani	0.516
CO 1	1.302
Ayali	0.834
Thilatara	1.335

4.3.2 Primer designing

Nucleotide sequences of the selected genes were retrieved from NCBI GenBank. ORFs of the sequence were identified using ORFfinder. Specific portion of the nucleotide that contain maximum ORFs were selected. Primer was designed for this region using Primer3 (Table 5). The primers were then synthesized at Sigma-Aldrich.

4.3.3 Amplification of gene of interest using PCR

PCR amplification revealed that both SSR markers were monomorphic in all the genotypes studied (Plates 12, 13). PCR amplification of *Phosphoenol pyruvate carboxylase* (Plate 14), *Galacturonate reductase* (Plate 15), *Inositol oxigenase* (Plate 16) and *Xyloglucan endotransglycosylase* (Plate 17) were also done.

4.3.4 DNA sequence analysis

Sequences obtained were analysed initially by BLASTn. The sequences of each gene and their BLASTn results are presented in Appendix I.

4.3.5 Sequence comparison using MAAFT

The DNA sequences of each gene in different genotypes were aligned and compared in MAAFT to identify any variations in nucleotide sequence (Fig.2-4).

4.3.6 Translation of DNA sequence using Expasy translate tool

Translated sequences are compared and the variations observed in the Open reading frames (ORFs) were identified. Variation are identified in *Phosphoenol pyruvate carboxylase* (Table 45) and *Xyloglucan endotransglycosylase* (Table 46).The variation that resulted in the insertion of stop codon can be considered as promising SNPs (Table 47)

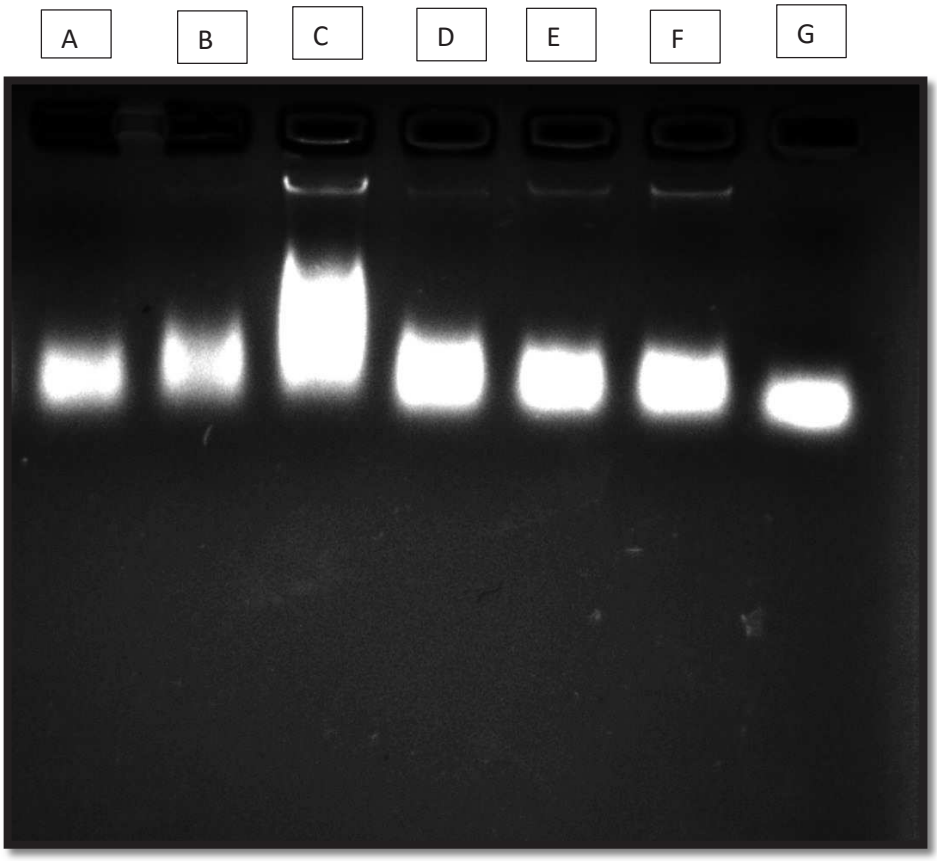


Plate 11. Integrity of the isolated DNA samples verified through electrophoresis

A. *Sesamum malabaricum,* B. TMV5, C. Kayankulam 1, D. Thilarani, E. CO 1, F Ayali, G. Thilatara

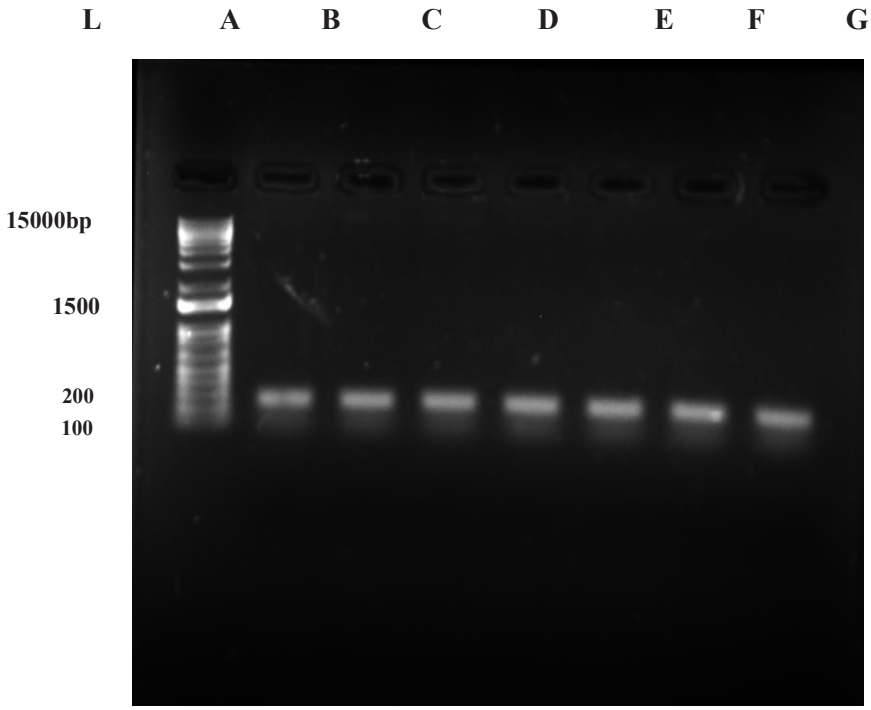


Plate 12. Gel documentation of PCR amplification SSR-ZM 22 marker from sesame genotypes. A. *Sesamum malabaricum,* B. TMV5, C. Kayamkulam 1, D. Thilarani, E. CO 1, F Ayali, G. Thilatara

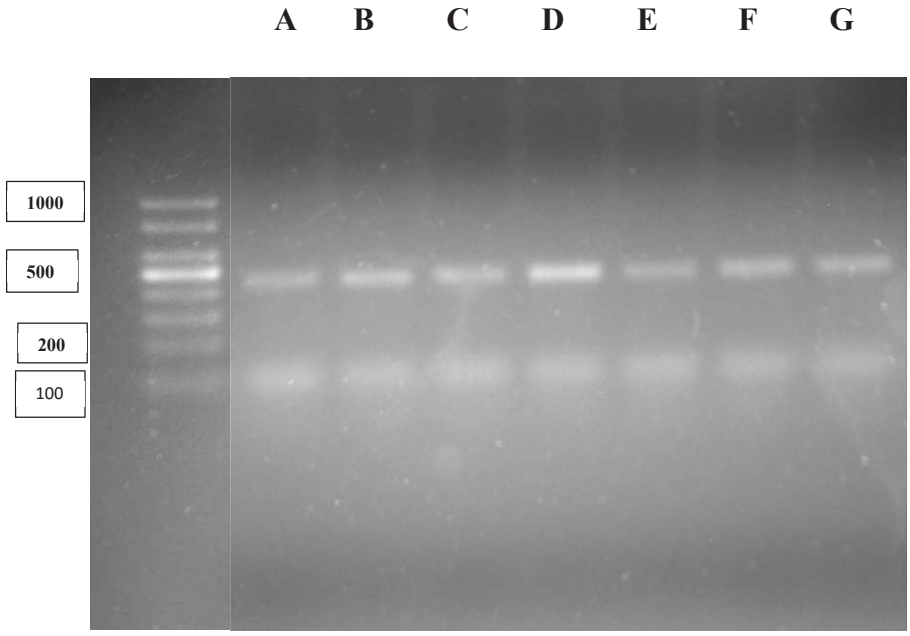


Plate 13. Gel documentation of PCR amplification of SSR-ZM428 marker from sesame genotypes. A. *Sesamum malabaricum,* B. TMV5, C. Kayamkulam 1, D. Thilarani, E. CO 1, F Ayali, G. Thilatara

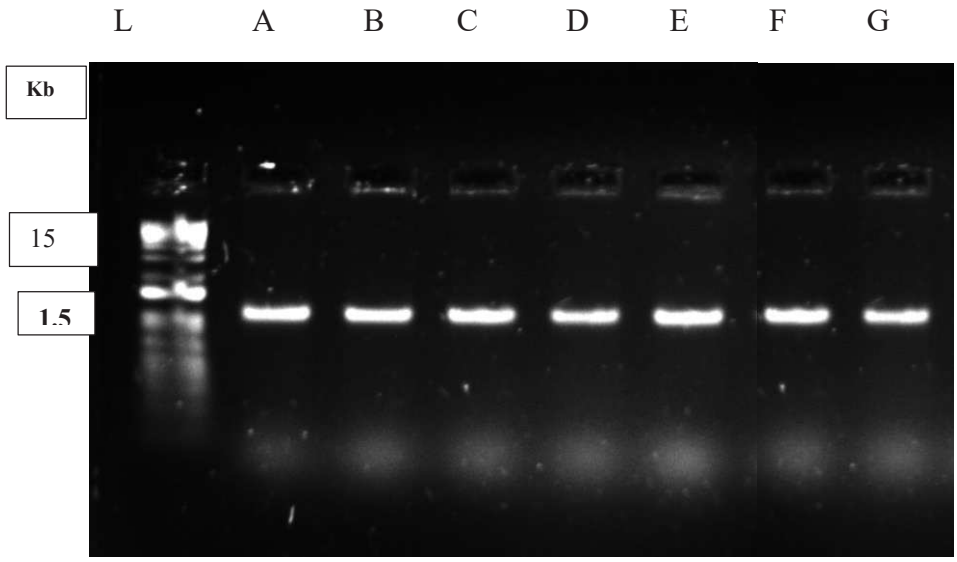


Plate 14. Gel documentation of PCR amplification of *Phosphoenol pyruvate carboxylase* gene from sesame genotypes. A. *Sesamum malabaricum,* B. TMV5, C. Kayamkulam 1, D. Thilarani, E. CO 1, F Ayali, G. Thilatara

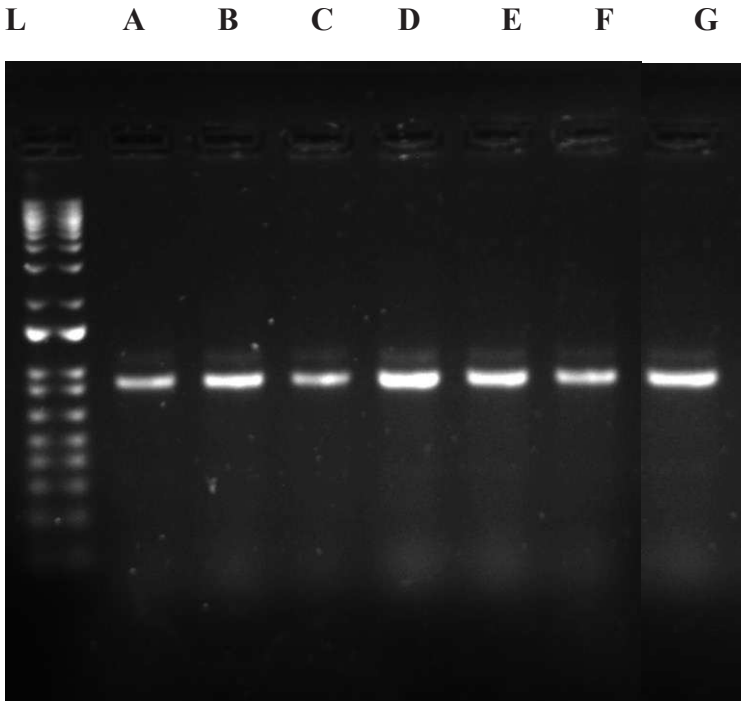


Plate 15. Gel documentation of PCR amplification of *Galacturonate reductase* gene from sesame genotypes. A. *Sesamum malabaricum,* B. TMV5, C. Kayamkulam 1, D. Thilarani, E. CO 1, F Ayali, G. Thilatara

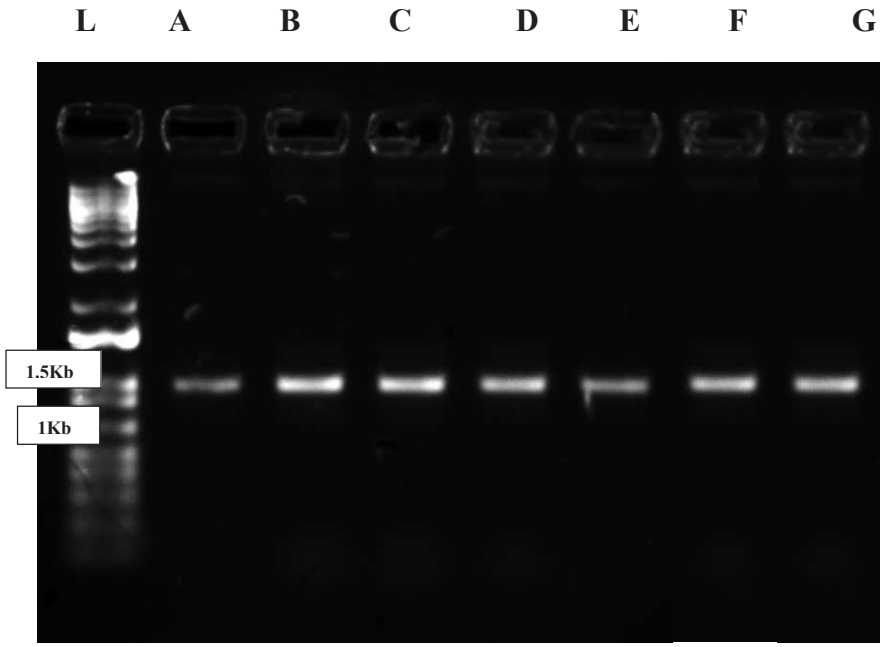


Plate 16. Gel documentation of PCR amplification of *Inositol oxigenase* gene from sesame genotypes. A. *Sesamum malabaricum,* B. TMV5, C. Kayamkulam 1, D. Thilarani, E. CO 1, F Ayali, G. Thilatara

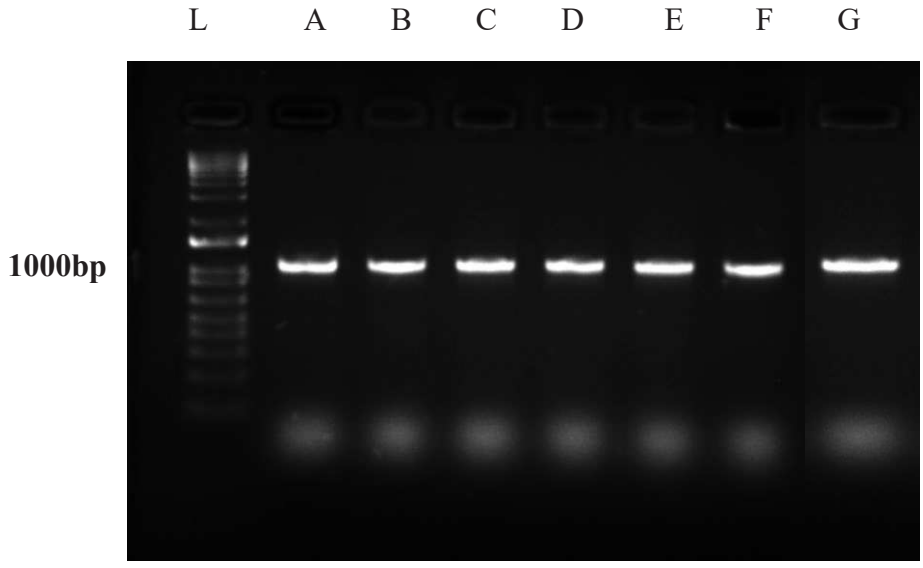


Plate 17. Gel documentation of PCR amplification *of Xyloglucan endotransglycosylase* gene from sesame genotypes. A. *Sesamum malabaricum,* B. TMV5, C. Kayamkulam 1, D. Thilarani, E. CO 1, F Ayali, G. Thilatara

Sequence comparison of *Phosphoenol pyruvate carboxylase*

Fig 2-. Sequence alignment *of Phosphoenol pyruvate carboxylase* from sesame genotypes in MAAFT

Sequence comparison of of *Xyloglucan endotransglycosylase carboxylase*

Fig 3. Sequences alignment of *Xyloglucan endotransglycosylase carboxylase* from sesame genotypes in MAAFT

Sequence comparison of *Galacturonate reductase*

Fig 4. Sequence alignment of *Galacturonate reductase* from sesame genotypes in MAAFT

Table 45. Variations identified in the nucleotide sequences of *Phosphoenol pyruvate carboxylase* in sesame var. Thilatara

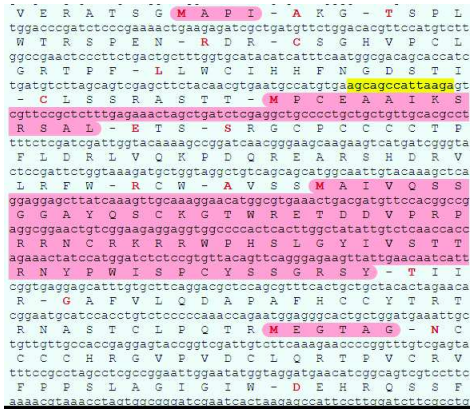
Variations	Observed change in amino acid
SNP of Adenine to Cytosine	Glutamic acid (E) to Aspartic acid (D) (Fig 5)
SNP of Guanine to Thymine	Alanine (A) to Serine (S) (Fig 6)
SNP of Thymine to Adenine	Tyrosine (Y) to stop codon (Fig 7)
Caatcattcg to t-cttttttt	Truncation of amino acid sequence (Fig 8)
gagcatttgtgct to tagaatttgagca	Truncation with stop codon (Fig 9)

Table 46. Variations identified in the nucleotide sequences of *Xyloglucan endotransglycosylase* in sesame var. CO 1

Variations	Observed change in amino acid
Insertion of adenine g-ggaactcctcc to gaggaactcctcc	Truncation of amino acid sequence (Fig 10)
Insertion of Cytosin g-atcggc to gcatcggc	Truncation of amino acid sequence (Fig 11)

Table 47. Promising SNPs identified

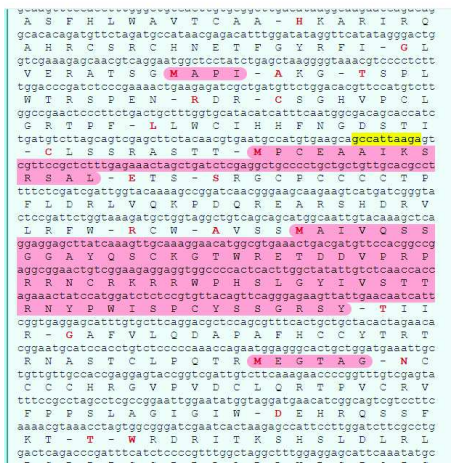
Gene	Genotype	SNP	Change in amino acid
Phosphoenol pyruvate carboxylase	Thilatara	SNP of Thymine to Adenine	Insertion of stop codon
Phosphoenol pyruvate carboxylase	Thilatara	gagcatttgtgct to tagaatttgagca	Insertion of stop codon



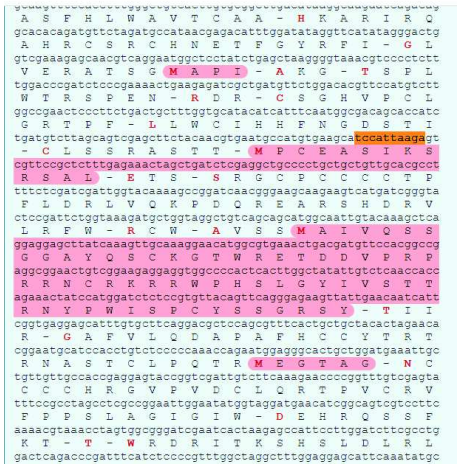
ORFs in moderately tolerant genotypes (Thilarani, Kayamkulam 1

ORFs in susceptible genotype Thilatara

Fig 5. SNP of Adenine to Cytosine results in change in amino acid of *Phosphoenol pyruvate carboxylase* in Thilatara

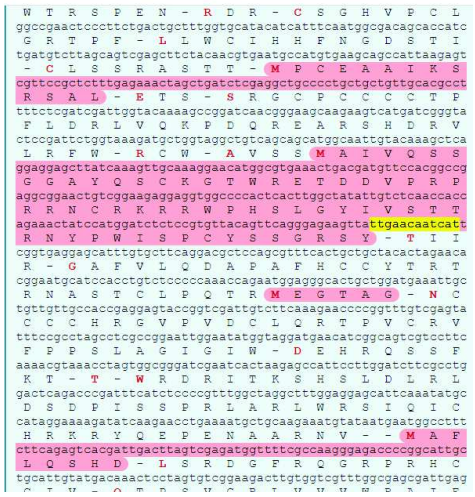


ORFs in moderately tolerant genotypes (Thilarani, Kayamkulam 1)

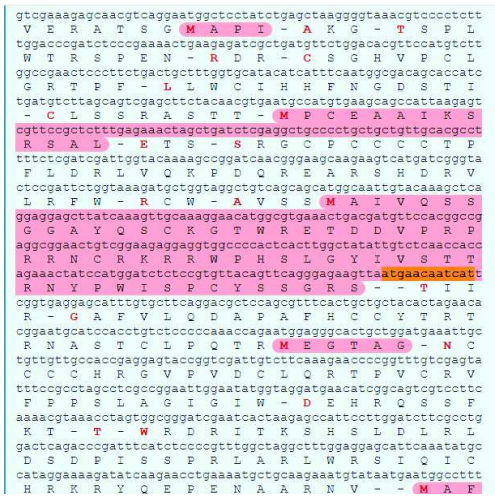


ORFs in susceptible genotype Thilatara

Fig 6. SNP of Guanine to Thymine results in change in amino acid of *Phosphoenol pyruvate carboxylase* in Thilatara

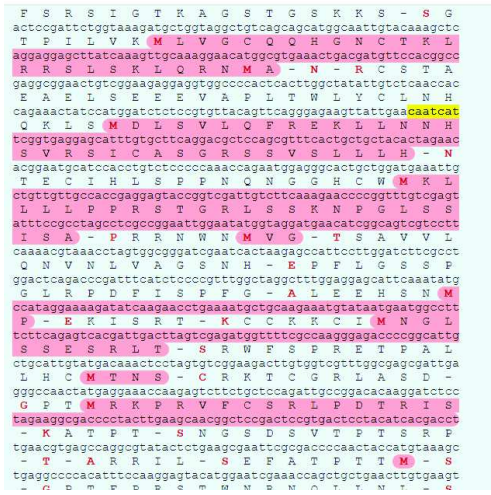


ORFs in moderately tolerant genotypes (Thilarani, Kayamkulam 1

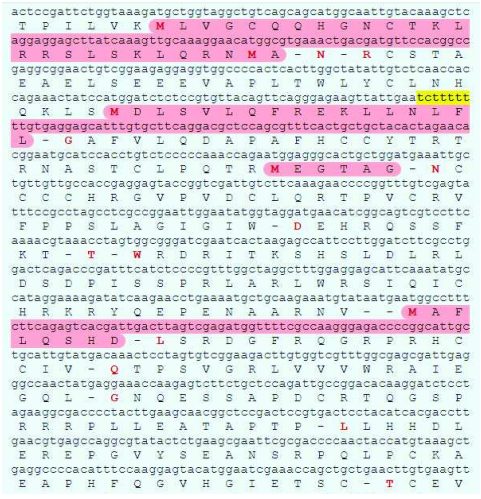


ORFs in susceptible genotype Thilatara

Fig 7. SNP of Thymine to Adenine results in insertion of stop codon in *Phosphoenol pyruvate carboxylase* in Thilatara

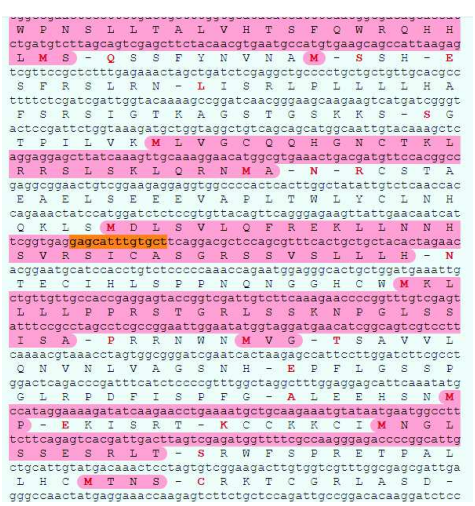


ORFs in moderately tolerant genotypes (Thilarani, Kayamkulam 1

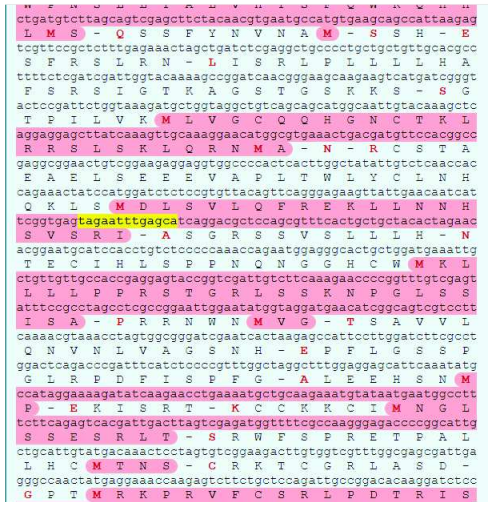


ORFs in susceptible genotype Thilatara

Fig 8. Variation in nucleotide sequence of *Phosphoenol pyruvate carboxylase* results in truncation of aminocid

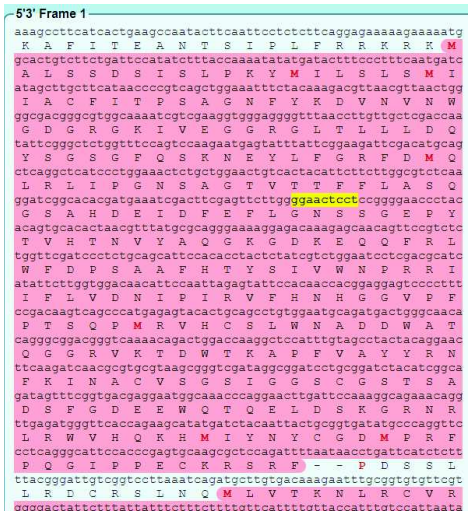


ORFs in moderately tolerant genotypes (Thilarani, Kayamkulam 1

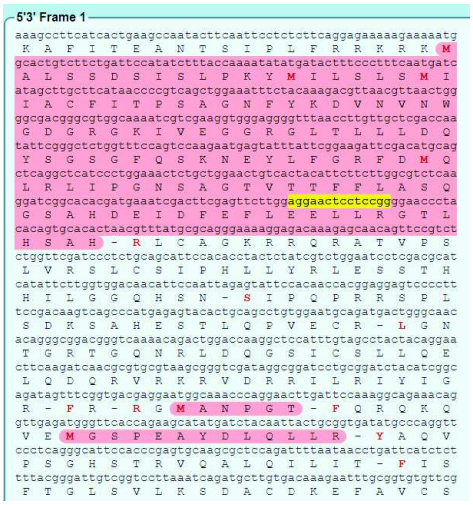


ORFs in susceptible genotype Thilatara

Fig 9. Variation in nucleotide sequence of *Phosphoenol pyruvate carboxylase* results in amino acid truncation with stop codon

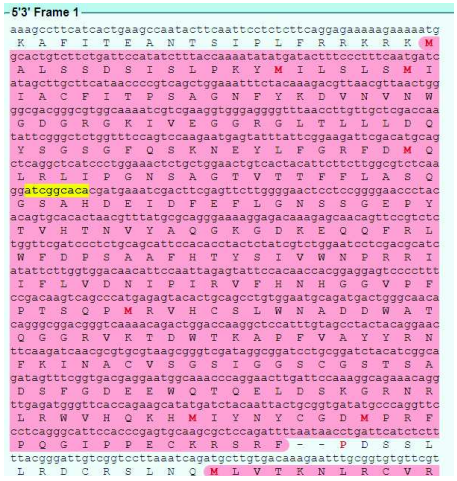


ORFs in tolerant genotype
S. malabaricum

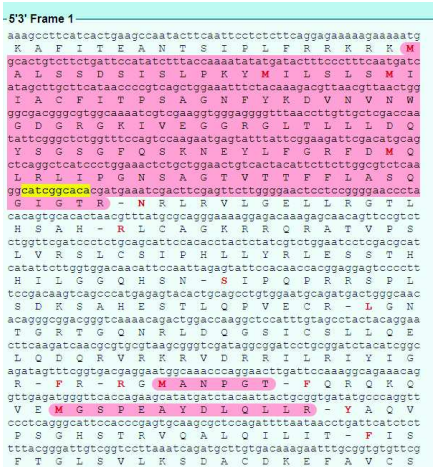


ORF in susceptible CO 1

Fig 10. Insertion of adenine in X*yloglucan endotransglycosylase* gene reults in truncation of aminoacid sequence



ORFs in tolerant genotype
S. malabaricum



ORF in susceptible CO 1

Fig 11. Insertion of cytosine in X*yloglucan endotransglycosylase* gene reults in truncation of aminoacid sequence

4.4 Amelioration of waterlogging stress (Waterproofing sesame) - Pot study

4.4.1 Morphological observations

4.4.1.1. Survival percentage (%)

Table 48 denotes the changes in survival per cent with the treatments. Among the 7 ameliorants, *P. fluorescens* (59.66 %), Salicylic acid (57.66 %), KNO_3 (55.75 %), Tricyclazole (52.50 %) and Urea (51.66 %) have improved the survival per cent of sesame plants compared to control 1 (45.38 %) plants, which recorded the lowest survival per cent which was statistically on par with NAA (48.66 %) and Ca $(NO_3)_2$ (49.5 %) treatments.

4.4.1.2. Plant height (cm)

Significant variation in plant height was observed with different treatments (Table 48). Control 2 (65.00cm) plants recorded the highest plant height. The lowest height was observed in control 1 (42.67cm) plants which was statistically on par with NAA (45.67cm), Tricyclazole (49.33 cm) and Urea (50.83 cm). All other treatments recorded higher plant height than control1.

4.4.1.3. Shoot dry weight (g)

Significant variation was observed in shoot dry weight with different treatments (Table 48).Among the treatments control 2 (0.560 g) recorded highest shoot dry weight followed by Salicylic acid (0.363 g), KNO_3 (0.354 g) and *P. fluorescens* (0.345 g). Control 1 (0.240g), tricyclazole (0.250g) and Urea (0.253g) recorded the lowest shoot dry weight as depicted in Table 45.

4.4.1.4. Root characters

The root dry weight (g), root length (cm) and root number were estimated from different treatments (Table 49). Highest root dry weight was observed in Tricyclazole (0.045g), *P. fluorescens* (0.045g) and Salicylic acid (0.044g) whereas the lowest in control 1 (0.015g). Root length was higher for control 2 (9.50cm) and lowest for NAA (3.30 cm), control 1 (4.00cm) and Tricyclazole (4.20cm). Salicylic acid (55.00), Tricyclazole (55.00), KNO_3 (50.66) and *P. fluorescens* (50.30) have recorded highest number of roots among all treatments. Lowest number of root was

Table 48. Effect of treatments on morphological parameters of sesame var. Thilak

Treatments	Survival per cent (%)	Plant height (cm)	Shoot dry weight (g)
T_1 : Urea	51.66^{cd}	50.83^{bcd}	0.253^{d}
T_2 : KNO_3	55.75^{bc}	55.33^{b}	0.354^{b}
T_3 : NAA	48.66^{de}	45.67^{cd}	0.157^{e}
T_4: $Ca(NO_3)_2$	49.75^{de}	53.00^{bc}	0.307^{c}
T_5 : *P. fluorescens*	59.66^{b}	55.33^{b}	0.345^{bc}
T_6 : Tricyclazole	52.50^{cd}	49.33^{bcd}	0.250^{d}
T_7 : Salicylic acid	57.66^{b}	54.07^{bc}	0.363^{b}
T_8 : Control 1	45.38^{e}	42.67^{d}	0.240^{d}
T_9 : Control 2	100.00^{a}	65.00^{a}	0.560^{a}
CD (0.05)	**4.80**	**8.66**	**0.05**

Table 49. Effect of treatments on root characters of sesame var. Thilak

Treatments	Root dry weight (g)	Root length (cm)	Root number per plant
T_1 : Urea	0.034^{d}	5.00^{bcd}	41.66^{b}
T_2 : KNO_3	0.042^{bc}	6.50^{b}	50.66^{a}
T_3 : NAA	0.040^{c}	3.30^{d}	31.33^{c}
T_4: $Ca(NO_3)_2$	0.023^{f}	4.70^{bcd}	39.66^{b}
T_5 : *P. fluorescens*	0.045^{a}	6.00^{bc}	50.30^{a}
T_6 : Tricyclazole	0.045^{a}	4.20^{cd}	55.00^{a}
T_7 : Salicylic acid	0.044^{ab}	6.30^{b}	55.00^{a}
T_8 : Control 1	0.015^{g}	4.00^{d}	41.33^{b}
T_9 : Control 2	0.027^{e}	9.50^{a}	37.33^{bc}
CD (0.05)	**0.003**	**1.94**	**6.27**

recorded in NAA (31.33) treatment which was statistically on par with control 2 (37.33).

4.4.2. Photosynthetic parameters

The data on photosynthetic parameters is given in Table 50. It is clear from the table that highest photosynthetic rate was obtained in control 2 (9.74 µ mole CO_2 $m^{-2}s^{-1}$) plants followed by KNO_3 (8.78 µ mole CO_2 $m^{-2}s^{-1}$) application. Among the 6 ameliorants tried, the application of KNO_3 (0.229 µ mole CO_2 $m^{-2}s^{-1}$) and salicylic acid (0.209 µ mole CO_2 $m^{-2}s^{-1}$) recorded highest stomatal conductance, whereas lowest in $Ca(NO_3)_2$ (0.083 µ mole CO_2 $m^{-2}s^{-1}$) application. Control 2 (7.487 µ mole CO_2 $m^{-2}s^{-1}$) plants followed by salicylic acid (5.530 µ mole CO_2 $m^{-2}s^{-1}$) and KNO_3 (5.207 µ mole CO_2 $m^{-2}s^{-1}$) application resulted in higher transpiration rate in plants while control 1 (1.333 µ mole CO_2 $m^{-2}s^{-1}$) followed by $Ca(NO_3)_2$ (2.253 µ mole CO_2 $m^{-2}s^{-1}$) application caused lower transpiration rate.

4.4.3. Biochemical parameters

4.4.3.1. Catalase enzyme activity (µ mol of H_2O_2 utilized $g^{-1}min^{-1}$)

The catalase enzyme activity was varied with treatments (Table 51). Salicylic acid (70.76 µ mol of H_2O_2 utilized $g^{-1}min^{-1}$) and *P. fluorescens* (70.44 µ mol of H_2O_2 utilized $g^{-1}min^{-1}$) application in plants recorded the highest catalase enzyme activity whereas control 1 (19.35 µ mol of H_2O_2 utilized $g^{-1}min^{-1}$) and control 2 (22.74 µ mol of H_2O_2 utilized $g^{-1}min^{-1}$) plants recorded the lowest and were statistically on par.

4.4.3.2 MDA content (nmol g^{-1})

The MDA content of various treatment application was given in Table 51. It is observed that control 1 (7.73 nmol g^{-1}) plants produced highest MDA while control 2 plants (3.63 nmol g^{-1}), the lowest. Whereas all other treatments produced lesser MDA than control 1.

4.4.3.3 Chlorophyll content (mgg^{-1})

Among the treatments control 2 followed by application of $Ca(NO_3)_2$ (0.91 mg g^{-1}), *P. fluorescens,* (0.90 mg g^{-1}), KNO_3 (0.81 mg g^{-1}) and salicylic acid (0.78 mg g^{-1}) resulted higher chlorophyll content whereas the control 1 (0.39 mg g^{-1}) recorded the lowest value (Table 51).

4.4.3.4 Nitrate reductase enzyme activity (µ moles of NO_2^- g^{-1} hr^-)

The highest nitrate reductase enzyme activity was obtained in control 2 (393.33 µ moles of NO_2^- g^{-1} hr^{-1}) plants followed by Urea (283.33 µ moles of NO_2^- g^{-1} hr^{-1}) and KNO_3 (206.66 µ moles of NO_2^- g^{-1} hr^{-1}) treatments. The lowest was recorded in control 1 plants (180.00 µ moles of NO_2^- g^{-1} hr^{-1}) (Table 51) (Table 51).

4.4.3.5 Soluble protein content (mg g^{-1})

As depicted in Table 51, Control 2 (35.50 mg g^{-1}) plants recorded highest soluble protein content. Application of *P. fluorescens* (27.25 mg g^{-1}) resulted in increase in soluble protein content than control 1 (17.50 mg g^{-1}), in contrast to other ameliorative treatments which were on par with control 1.

Table 50. Effect of treatments on photosynthetic parameters of sesame var. Thilak

Treatments	Photosynthetic rate (µ mole CO_2 $m^{-2}s^{-1}$)	Stomatal conductance (µmol $m^{-2}s^{-1}$)	Transpiration rate (µmol H_2O $m^{-2}s^{-1}$)
T_1: Urea	6.75^{d}	0.134^{c}	3.427^{f}
T_2: KNO_3	8.78^{b}	0.229^{b}	5.207^{c}
T_3: NAA	2.07^{f}	0.136^{c}	2.880^{g}
T_4: $Ca(NO_3)_2$	1.43^{h}	0.083^{d}	2.253^{h}
T_5 : *P. fluorescens*	7.15^{c}	0.159^{c}	4.697^{d}
T_6: Tricyclazole	1.79^{g}	0.143^{c}	3.463^{e}
T_7: Salicylic acid	6.55^{e}	0.209^{b}	5.530^{b}
T_8: Control 1	1.12^{i}	0.047^{d}	1.333^{i}
T_9: Control 2	9.74^{a}	7.487^{a}	14.800^{a}
CD (0.05)	**0.067**	**0.036**	**0.008**

Table 51. Effect of treatments on biochemical parameters of sesame var. Thilak

Treatments	Catalase enzyme activity (µ mol of H_2O_2 utilized $g^{-1}min^{-1}$)	MDA content (nmol g^{-1})	Total chlorophyll content (mg g^{-1})	Nitrate reductase enzyme activity (µ moles of NO_2^- formed g^{-1} hr^{-1})	Soluble protein content (mg g^{-1})
T_1: Urea	48.56^{d}	6.02^{c}	0.71^{cd}	283.33^{b}	14.37^{e}
T_2: KNO_3	64.08^{b}	5.22^{d}	0.81^{bc}	206.66^{c}	18.12^{cd}
T_3: NAA	55.83^{c}	6.70^{b}	0.71^{cd}	100.00^{f}	14.87^{de}
T_4: $Ca(NO_3)_2$	59.08^{c}	6.22^{bc}	0.91^{b}	200.00^{cd}	18.37^{cd}
T_5 : *P. fluorescens*	70.44^{a}	4.48^{e}	0.90^{b}	210.00^{c}	27.25^{b}
T_6: Tricyclazole	50.33^{d}	6.30^{bc}	0.55^{d}	195.00^{d}	14.50^{e}
T_7: Salicylic acid	70.76^{a}	5.28^{d}	0.78^{bc}	203.33^{cd}	19.00^{c}
T_8: Control 1	19.35^{e}	7.73^{a}	0.39^{e}	180.00^{e}	17.50^{cde}
T_9: Control 2	22.74^{e}	3.63^{f}	1.19^{a}	393.33^{a}	35.50^{a}
CD (0.05)	**4.46**	**0.69**	**0.16**	**10.44**	**3.584**

4.5. RESULTS OF FIELD STUDY

A field experiment was conducted to study the effect of ameliorants on water proofing of sesame variety Thilak. Best three ameliorants selected from pot culture study was used in the field. Morphological and yield parameters were recorded. The results of the study are detailed below.

4.5.1 Morphological observations

4.5.1.1 Survival percentage (%)

Effect of treatments on plant survival under waterlogging is given in Table 52. All the three ameliorants significantly improved the survival percentage of sesame. Among them, KNO_3 recorded highest survival (90.80%) followed by *P. fluorescens* (84.74), whereas the lowest was observed in control 1 plots (68.66%). The treatment with salicylic acid (81.42 %) also recorded higher survival than control 1 plants.

4.5.1.2 Plant height (cm)

Waterlogging stress significantly reduced the plant height of sesame plants as evident from lowest plant height recorded in control 1 (111.33 cm), while highest plant height was recoded in Control 2 plants (137.33 cm), where no waterlogging and no ameliorants were given (Table 52). All the three ameliorants significantly improved the plant height compared to control 1 with highest being observed in *P. fluorescens* (125.17 cm) and KNO_3 (122.50 cm) treated plots.

4.5.1.3 Shoot dry weight (g)

The data in Table 52 indicates the positive influence of ameliorants on biomass production under waterlogged condition. The non-waterlogged control 2 (30.23g) plants recorded highest shoot dry weight followed by KNO_3 (25.76 g) and *P. fluorescens* (26.00g) which were statistically on par. Plants in waterlogged condition (control 1) without ameliorant sprays (19.33g) recorded the lowest value.

Table 52. Effect of treatments on morphological observarions of sesame var. Thilak

Treatments	Survival per cent (%)	Plant height at harvest (cm)	Shoot dry weight at harvest (g)	Root dry weight at harvest (g)	Leaf number	Days to 50 % flowering
T_1 : KNO_3	90.80^{b}	122.50^{bc}	25.76^{b}	3.34^{c}	39.33^{b}	37.25
T_2 : *P. fluorescens*	84.74^{c}	125.17^{b}	26.00^{b}	4.13^{b}	41.67^{b}	37.00
T_3 : Salicylic acid	81.42^{d}	119.50^{c}	24.00^{c}	3.50^{c}	36.67^{bc}	40.75
T_4 : Control 1	68.66^{e}	111.33^{d}	19.33^{d}	2.46^{d}	32.00^{c}	37.75
T_5 : Control 2	100.00^{a}	137.33^{a}	30.23^{a}	4.66^{a}	51.00^{a}	41.50
CD (0.05)	**3.28**	5.3	1.49	0.41	5.964	NS

4.5.1.4 Root dry weight (g)

Waterlogging significantly reduced the root dry weight of sesame plants (2.46g) as compared to non-waterlogged condition (4.66g). Ameliorant application significantly improved the root biomass production as seen in Table 52. Among the three ameliorants, highest root dry weight was seen in *P. fluorescens* (4.13g) followed by salicylic acid (3.50g) and KNO_3 (3.34g) which were statistically on par.

4.5.1.5 Leaf number

The leaf number per plant of different treatment plots are given in Table 52. Control 1 (32.00) recorded the lowest leaf number which was statistically on par with salicylic acid treatment (36.67). Control 2 (51.00) recorded highest leaf number followed by *P. fluorescens* (41.67) and KNO_3 (39.33) which were statistically on par.

4.5.1.6 Days to 50 per cent flowering

Waterlogging during the initial growth stages did not have any significant influence on days to 50 per cent flowering in sesame var. Thilak as depicted in Table 52.

4.5.2 Yield and yield attributes

4.5.2.1 No. of branches per plant

Branching in sesame was significantly affected by waterlogging (Table 53). Highest branch number was observed in control 2 (6.67), whereas lowest from control 1 (4.00) (Table 53). All the three ameliorants *viz*, *P. fluorescens* (5.33), KNO_3 (5.00) and Salicylic acid (5.00) recorded statistically similar values and were significantly higher than Control 1 (4.00).

4.5.2.2. Number of capsules per plant

Among the treatments, control 2 (77.33), which was not subjected to waterlogging recorded the highest number of capsules per plant followed by *P. fluorescens* (67.66) and KNO_3 (66.00) which were statistically on par. The lowest was recorded from control 1 (58.66) plots (Table 53).

Table 53. Effect of treatments on yield and yield attributes of sesame var. Thilak

Treatments	No. of branches per plant	No. capsules per plant	No. seeds per capsule	Thousand seed weight (g)	Yield per plant (g)	Yield (t ha^{-1})
T_1 : KNO_3	5.00^{b}	66.00^{bc}	54.00^{bc}	3.05	5.30^{b}	0.795^{b}
T_2 : *P. fluorescens*	5.33^{b}	67.66^{b}	56.66^{ab}	2.98	5.43^{b}	0.815^{b}
T_3 : Salicylic acid	5.00^{b}	64.00^{c}	53.33^{c}	2.62	5.03^{c}	0.755^{c}
T_4 : Control 1	4.00^{c}	58.66^{d}	50.00^{d}	2.48	4.66^{d}	0.70^{d}
T_5 : Control 2	6.67^{a}	77.33^{a}	59.00^{a}	2.78	6.06^{a}	0.91^{a}
CD (0.05)	0.643	3.197	3.046	NS	0.150	0.022

4.5.2.3 Number of seeds per capsule

Highest number of seeds per capsule was recorded in control 2 (59.00) plants, which was statistically on par with *P. fluorescens* (56.66). Plants sprayed with KNO_3 (54.00) and salicylic acid (53.33) also recorded higher number of seeds than T_4 (Table 53).

4.5.2.4 Thousand seed weight (g)

Thousand seed weight was not influenced by water logging during the initial growth stages of the crop. The treatment effect was observed to be non- significant as depicted in Table 53.

4.5.2.5 Yield per plant (g) and Yield per hectare (t ha^{-1})

Waterlogging significantly reduced the per plant yield of sesame (Table 53). The lowest yield was recorded in T_4 (4.66g), while highest yield was recorded in T_5 (6.06g), where no waterlogging was imposed. Ameliorant sprays significantly improved the per plant yield. Application of *P. fluorescens* (5.43g) and KNO_3 (5.30g) recorded statistically on par values. Salicylic acid treatment (5.03g) recorded statistically lower yield than *P. fluorescens* and KNO_3, while statistically higher yield than T_4. Yield per plant and yield per ha recorded were in a similar pattern (Table 53).

Discussion

5. DISCUSSION

Sesame (*Sesamum indicum* L.) is an ancient oil crop widely cultivated in many parts of the world, and it meets the need for high quality oil. The present study identifies the constraints faced by sesame farmers in Kerala which has contributed to a decline in production of the crop in the state. It also focuses on long term and short term ameliorative measures that can be adopted for improving production and productivity of the crop. This includes screening of sesame genotypes for tolerance to waterlogging based on morpho-physiological characters, screening of sesame genotypes for drought tolerance at seedling stage, waterproofing studies using chemical ameliorants for waterlogging which is a major constraint and molecular characterization of genotypes for inclusion in breeding programmes.

5.1 FARMER SURVEY

A survey conducted among the sesame farmers of Kollam, Alapuzha and Thrissur districts indicated that the constraints faced by them were high labour cost, excessive rain, drought, weed infestation, labour unavailability, pest and diseases, marketing, transportation, drying and threshing and storage problems (Fig.12). Providing appropriate suggestion is important as our state has been witnessing a declining trend in sesame production. The suggestions offered by farmers and scientists and those collected from literatures to solve the above mentioned constraints are enumerated below.

During the recent years excessive and untimely rain fall has posed a major problem in sesame cultivation. Water proofing the crop is an urgent requirement for enhancing cultivation. This can be with the use of chemical ameliorants or by developing waterlogging tolerant varieties. Athul (2016) identified a local sesame variety ‘Ayaly’ showing tolerance to waterlogging. Other suggestion offered for dealing with the problem includes the application of foliar fertilizers (Pang *et al*., 2007), use of slow-release or controlled-release fertilizers (Varadachari and Goertz, 2010) and suitable plant growth regulators (Ren *et al*., 2018b). Installing a proper drainage system. Incorporating herbaceous perennial legumes such as lucerne, clovers and Messina (*Melilotus siculus*) adapted to waterlogging and inundation,

along with the main crop has been suggested to reduce waterlogging (Cocks, 2001).However some of these suggestion are not applicable in Kerala as sesame is grown as an additional crop in summer rice fallows.

Sesame is mainly grown in summer as a rainfed crop in Kerala. It is considered to be more drought resistant than many other crops. However during the plant establishment phase it is susceptible to moisture stress. Poor germination and slow growth are major problems under drought. Seed priming can be practiced to improve tolerance to drought in field condition. Hydropriming and osmopriming can be practiced to induce drought tolerance (Farooq and Hussain, 2017). Increasing soil organic matter with organic fertilizers or green manure (Bond and willis, 1969), mulching, use of antitranspirants, reducing plant density and competition among plants, use of phosphatic fertilizers that promote radical growth and use of tolerant varieties can be adopted.

Hand weeding is the only practiced method of weed control in sesame by the farmers. Use of stale seed bed method and application of preemergence herbicides can control weeds to a major extent. Other than depending on chemical herbicides, weed suppression can be achieved by improving early vigour of sesame plants through seed priming techniques such as hydropriming and nutrient priming (Sreepriya and Girija, 2018). Weed density and biomass in sesame field could be significantly suppressed by mulching with wheat straw (Al-Eqaili, 2017). Similarly, rice straw can be used as a cover crop in sesame field in Kerala. These practices also limits labour cost and need of labour.

High labour cost is the major constraint of sesame farmers in Kerala. Due to lack of mechanization and unavailability of labour they have to depend on labours from other states, contributing to additional labour cost. According to farmer's suggestion, cultivating sesame in our own homesteads without employing labour is the profitable way of sesame cultivation. Lack of proper market is a constraint for large scale producers and it should be solved at the earliest.

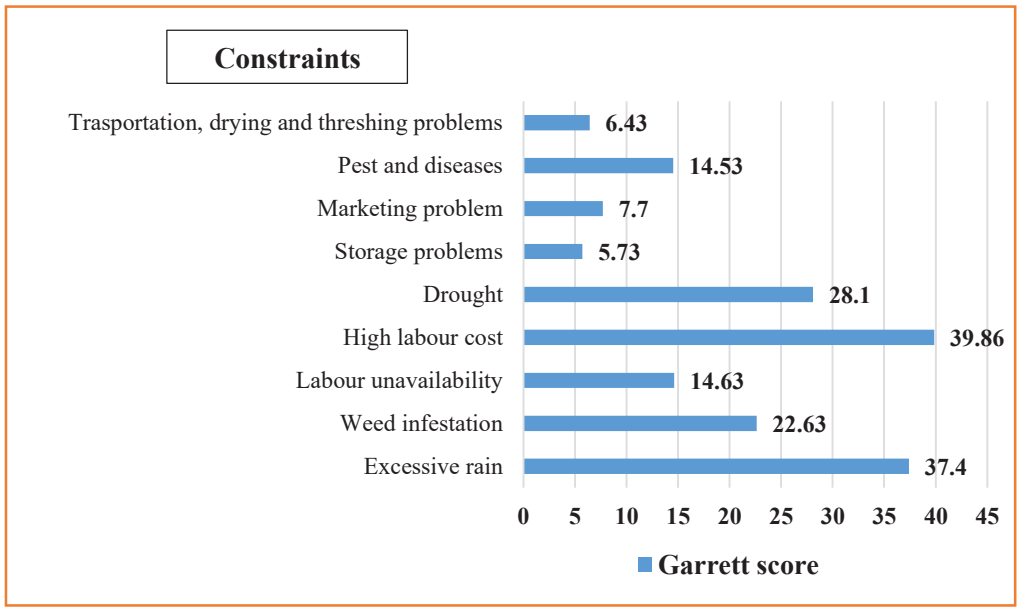


Fig 12. Constraints faced by sesame growing farmers of Kerala using Garrett ranking

5.2 SCREENING FOR TOLERANCE TO WATERLOGGING

Waterlogging studies with 15 sesame genotypes identified that there is significant difference in morphological response among them. In the genotypes *S. malabaricum,* Ayali and SVPR 1, no wilting was noted at the end of waterlogging. Lower leaf wilting and yellowing was observed in TMV 5, TMV7, Thilarani and Thilak (Plate 18). All others showed leaf wilting, the severity varied with genotype. CO1 and TMV 4 were the first ones to show symptoms of wilting (Plate 19).

5.2.1. Effect of waterlogging on morphological characters

5.2.1.1 Survival percentage

Significant variation in survival percentage under waterlogged condition was observed in sesame genotypes (Fig. 13). At both stages of waterlogging, *S. malabaricum* possessed highest survival of 100 per cent. At vegetative stage, more than 70 per cent survival was recorded in the genotypes, Ayali, Thilarani and SVPR1. The genotypes CO1, Thilatara and AT231 recorded less than 40 per cent survival. Under reproductive stage, all genotype possessed 100 per cent survival except CO1. This was in agreement with Zhou (2014), whose study in rapeseed revealed that good tolerance at seedling stage can guarantee tolerance in later stages of plant growth.

5.2.1.2 Plant height and shoot dry weight

Plant height and shoot dry weight are major vegetative characters that decide productivity of sesame. The study indicated that water logging at both stages affected the growth of the plant in all the 15 genotypes which was in conformity with the findings of Sarkar *et al*. (2016) and Hossain (2006) in sesame. Inundation of the root system might have resulted in poor energy status of the crop which in turn contributed to low biomass production (Wei *et al*., 2013). At vegetative stage, *S. malabaricum* recorded least reduction in plant height (Table 8) and shoot dry weight (Table 16) whereas CO1 recorded highest reduction of both characters. At reproductive stage, all genotypes except CO 1 showed less than 20 per cent reduction in plant height (Table 9).

5.2.1.3 Root characters

Estimation of root morphological parameters such as root length, number and dry weight indicated that the most important parameter under waterlogging is the root number. At vegetative stage in three genotypes *viz*., CO1, Thilatara and AT231, the root number decreased while in all other genotypes an increase was noted (Table 12). This was more pronounced in the genotypes, Thilarani, Ayali, *S. malabaricum* and TMV 5 when water logging was in the vegetative stage. The formation of adventitious roots in the genotype Ayali is given in Plate 20. Root morphology of sesame genotypes waterlogged are given in Plate 21-27. This increase in root number is due to the increased production of adventitious roots which can be regarded as potential morphological adaptations depicted by plants under waterlogging stress (Malik *et al*., 2001). This was in agreement with the finding of Wei *et al*. (2013), who observed an increase in root number in waterlogging tolerant sesame genotype (ZZM2541) while decrease in root number in waterlogging indicated susceptible genotype (Ezhi-2). When waterlogging was in the flowering stage, the root number was not affected in all the sesame genotypes.

5.2.2 Biochemical changes in leaf

Waterlogging results in different biochemical alterations in plant leaves. Changes in major biochemical constituents such as Chlorophyll content, Nitrate reductase enzyme, soluble protein content were estimated. Lipid peroxidation and ROS scavenging which are the important indicators for stress tolerance was estimated as MDA content and catalase enzyme activity. The changes in these parameters in leaves were estimated immediately after waterlogging and in non-waterlogged plants.

Waterlogging resulted in reduction of chlorophyll content in all genotypes at both stages (Fig.14). The reduction of chlorophyll content in leaves can be justified as reported by Ren *et al*. (2016) in Maize, that waterlogging damages the chloroplast

Plate 18 A. *S. malabaricum*

Plate 18 B. Ayali

Plate 18 C. TMV 7

Plate 18 D. TMV 5

Plate 18 E. Thilarani

Plate 18 F. Thilak

Plate 18. Morphological responses of sesame genotypes with higher survival under waterlogging. *S. malabaricum* (A), Ayali (B), TMV 7(C) and TMV 5(D) and Thailarani (E) at the end of waterlogging at vegetative stage

Plate 19 A. CO 1

Plate 19 B. TMV4

Plate 19 C. TMV3

Plate 19. Morphological response of sesame genotypes with lower survival under waterlogging

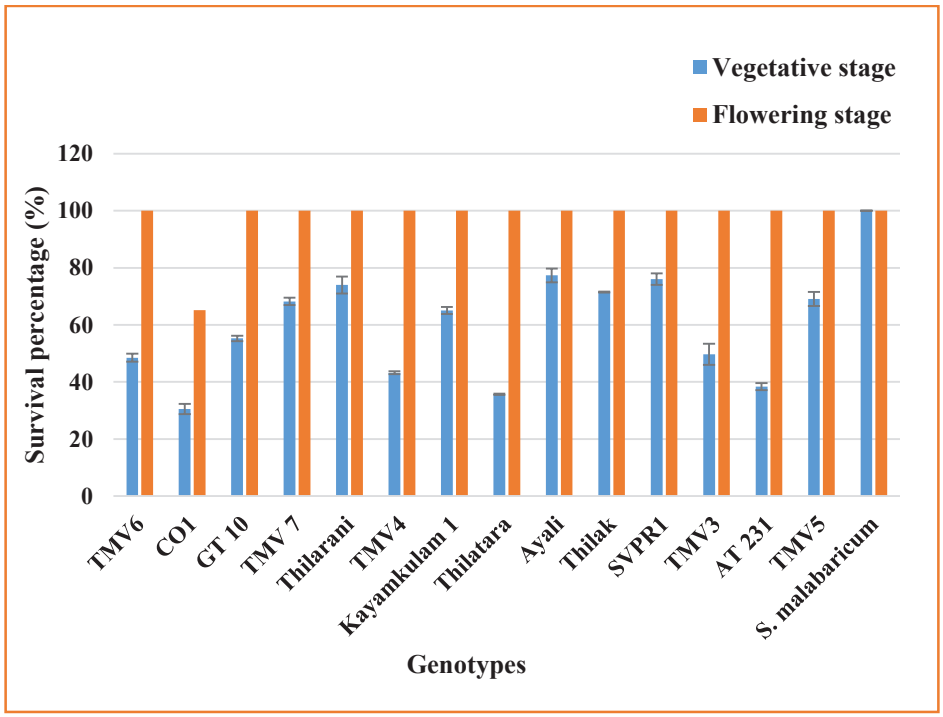


Fig 13. Survival percentage of sesame genotypes waterlogged at vegetative and flowering stage

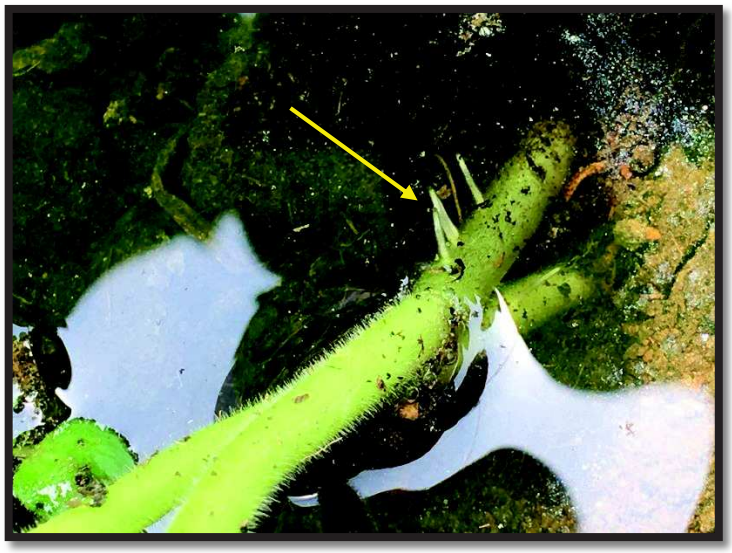


Plate 20. Production of adventitious roots in sesame var. Ayali in one day after waterlogging

Plate 21. Root morphology of *sesamum malabaricum* in waterlogged and control condition

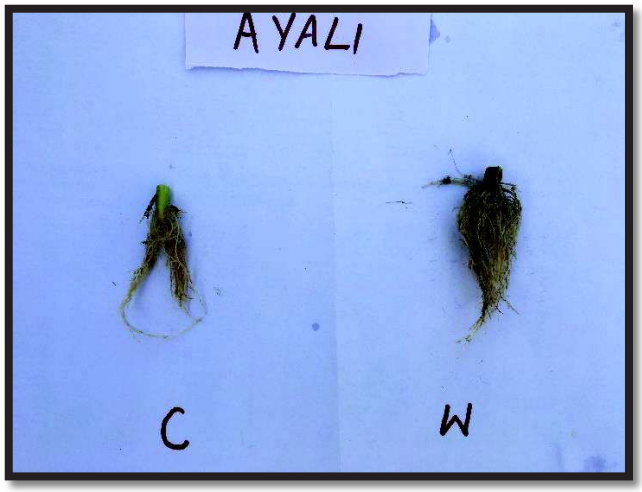


Plate 22. Root morphology of sesame genotype Ayali in waterlogged and control condition

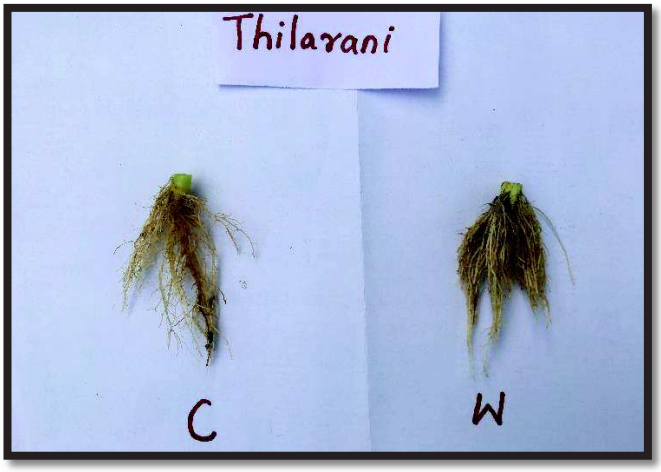


Plate 23. Root morphology of sesame genotype Thilarani at waterlogged and control condition

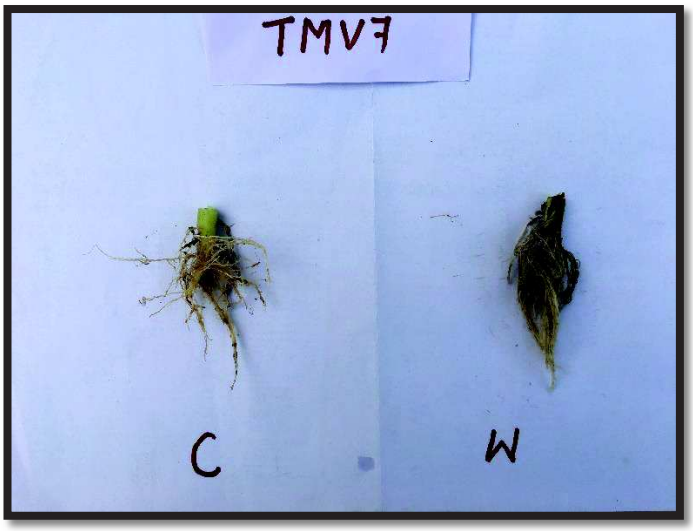


Plate 24. Root morphology of sesame genotype TMV 7 at waterlogged and control condition

Plate 25. Root morphology of sesame genotype TMV 5 at waterlogged and control condition

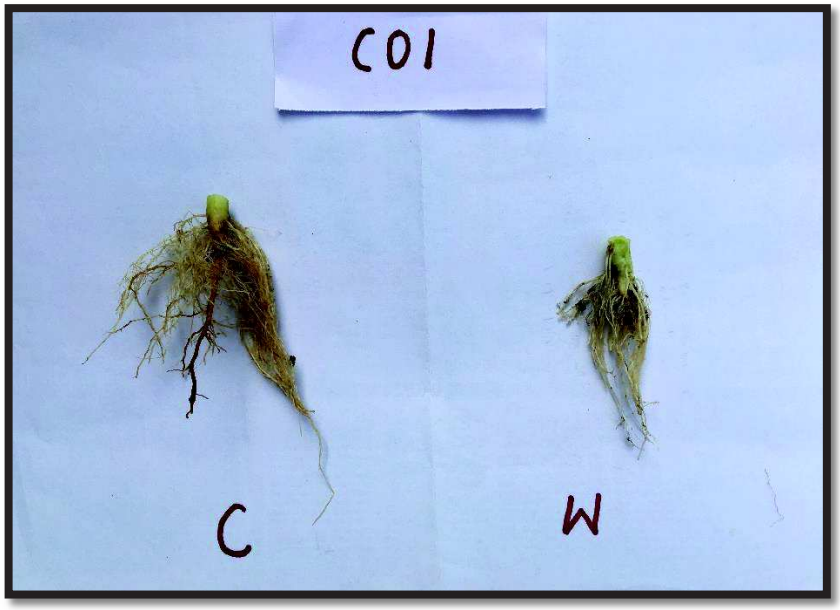


Plate 26. Root morphology of sesame genotype CO 1 at waterlogged and control condition

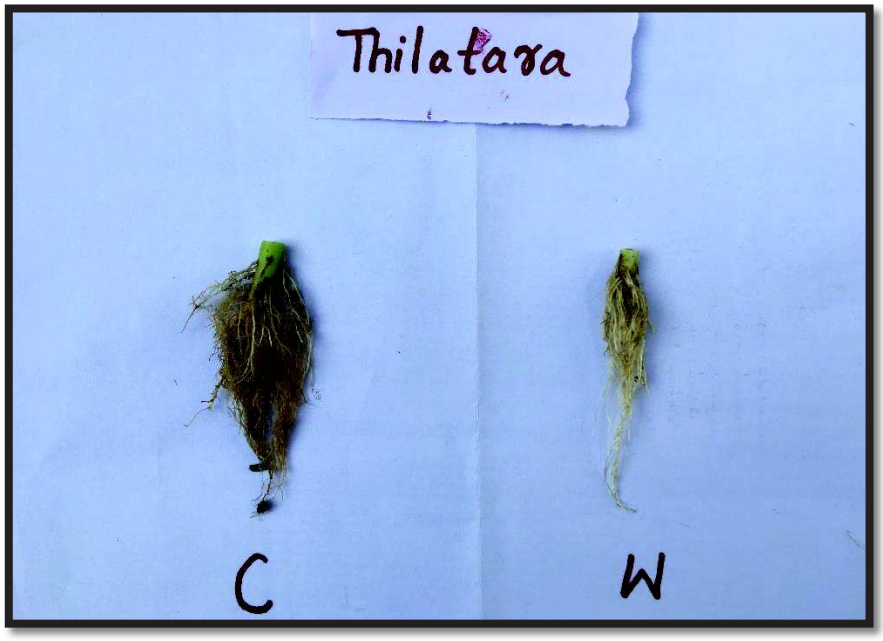


Plate 27. Root morphology of sesame genotype Thilatara in control and waterlogged condition

morphology and ultrastructure of functional leaves. The reduction in chlorophyll content varied among genotypes. The chlorophyll content was more affected at vegetative stage (up to 32%) than reproductive stage (up to 14%) (Fig 14). Thilatara recorded highest reduction whereas, Thilarani and *S. malabaricum* recorded least reduction in chlorophyll content. Wei *et al*. (2013) reported least decrease in chlorophyll in waterlogging tolerant and higher reduction in waterlogging sensitive sesame genotypes.

Nitrate reductase enzyme activity was reduced under waterlogging in both stages. This can be due to the reduced nitrate import from root to shoot as reported by Sepehr *et al*. (2012) in cumin. Least reduction in NRase activity was estimated in Ayali, Kayamkulam 1, Thilarani and *S. malabaricum* (Table 26) which implied that in these genotypes, nitrogen translocation from root to shoot was not much affected as compared to other genotypes. Highest reduction was recorded in the genotype Thilatara, CO1 and AT231 at vegetative stage (Table 26) and CO 1 at reproductive stage (Table 27).

Wu *et al*. (2003) suggested that malondialdehyde (MDA) produced as a byproduct of lipid peroxidation by ROS as a general indicator for water logging tolerance in plants. In the present study, MDA content increased in all genotypes imposed with waterlogging at both stages attributed by enhanced production of ROS under stress condition. At vegetative stage *S.malabaricum*, Ayali, Kayamkulam 1 and Thilarani recorded least increase in MDA content, while Thilatara and CO1 recorded the highest increase (Fig 15). This finding is similar to the report of Xu *et al*. (2012), who observed more increase in MDA content in susceptible sesame variety than in tolerant varieties (WTG-2541, WTG- 2413) under waterlogging. At reproductive stage (Table 21), MDA production was comparatively low as compared to vegetative stage. The per cent increase in MDA content was also less (less than 35 %) (Table 21). In *S. malabaricum* the percentage increase in MDA content at flowering stage was not least as in vegetative stage. This can be due to the effect of environmental condition at the time of experiment and also its behavioural response in different developmental stages. However, the percentage increase of MDA under

flowering stage was less than percentage increase in vegetative stage in *S. malabaricum*.

Catalase (CAT) is an enzyme which is regarded as an efficient ROS detoxifier (Hassanuzzaman *et al*., 2012). At vegetative stage catalase enzyme activity was increased in all genotypes except AT 231, TMV6, CO 1 and TMV4 (Table 22). At reproductive stage all genotypes possessed increased level of catalase enzyme activity (Table 23). Saha *et al*. (2016) also observed an increase in catalase enzyme activity under waterlogged condition in moderately tolerant sesame genotypes (BD 6980, BD 6992, BD 7012).

At vegetative stage all genotypes showed decline in total soluble protein content after waterlogging (Table 24). TMV5, Thilak, Thilarani, Ayali and TMV3 recorded least reduction over control, whereas TMV4, TMV6 and CO 1 recoded highest decline (Table 24). Similar results was recorded by Athul (2016) who studied waterlogging tolerance in sesame genotypes. Anaerobiosis has been reported to cause repression of normal protein synthesis (Sachs *et al*., 1980; Sinha *et al*., 1995). The ROS generated under waterlogged condition can also be detrimental to protein (Biemelt *et al*., 2000). At reproductive stage, no significant variation in total soluble protein content was observed with waterlogging (Table 25). More tolerance to waterlogging at this stage together with high antioxidant system such as catalase must have protected the protein content of seame genotypes during waterlogging.

5.2.3 Anaerobic enzyme activity in root

It has been reported that under waterlogged condition, which creates a hypoxic condition in root zone, there occur a shift from aerobic to anaerobic respiration in plant roots. During this fermentative glycolysis, anaerobic proteins such as pyruvate decarboxylase (PDC), alcohol dehydrogenase (ADH) and lactate dehydrogenase (LDH) are reported to be induced.

In this present study the activity of the above three anaerobic enzymes under waterlogged condition was estimated in 4 genotypes which showed higher survival

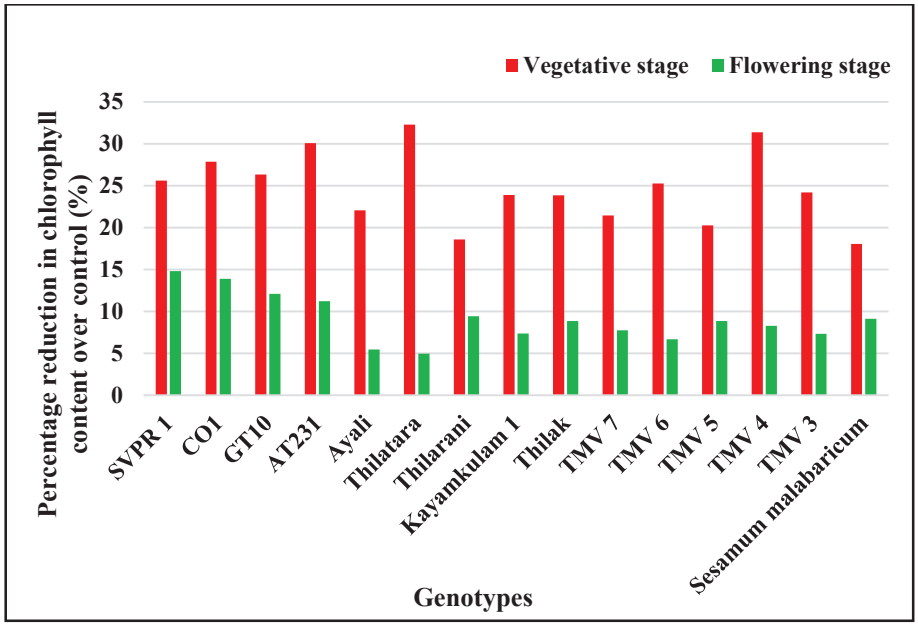


Fig 14. Percentage reduction over control in chlorophyll content of sesame genotypes waterlogged at vegetative and flowering stage

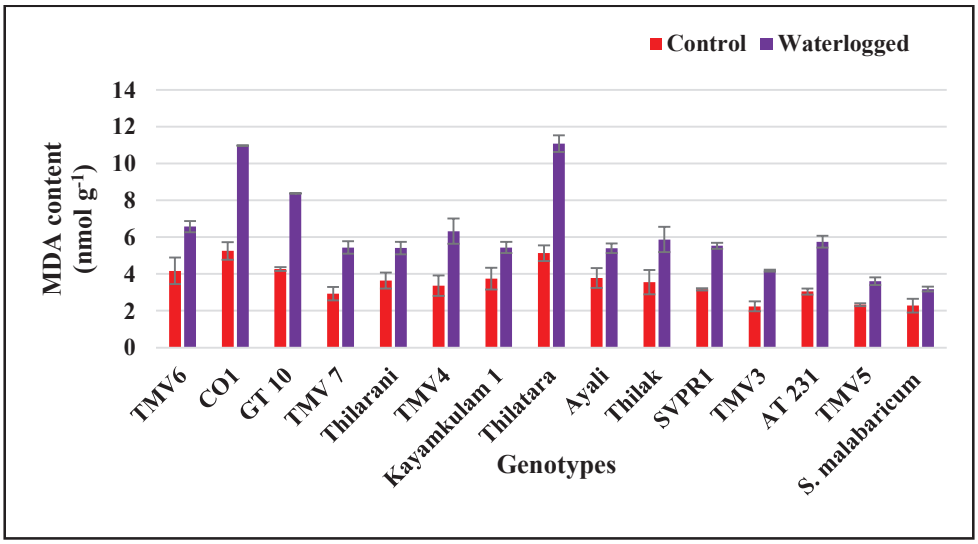


Fig 15. MDA content of sesame genotypes under control and waterlogged (vegetative stage) condition

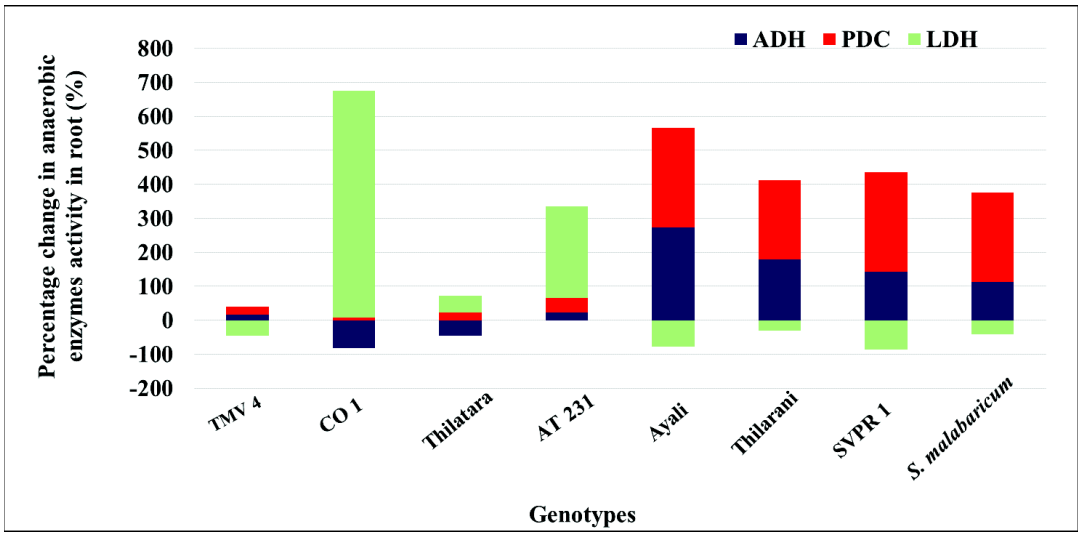


Fig 16. Percentage change in the activities of ADH, PDC and LDH over control in waterlogged sesame genotypes

(*S. malabaricum*, Ayali, Thilarani and SVPR1) and 4 genotypes which showed lower survival (CO1, AT231, Thilatara, TMV4).

In the genotypes CO 1, Thilatara and AT231, LDH enzyme activity was increased whereas in Ayali, Thilarani, *S. malabaricum* and SVPR 1 increase was noted in the activity of ADH and PDC (Fig. 16). This indicate that lactic acid fermentation was predominant than alcoholic acid fermentation in Thilatara, CO1 and AT231. The lactic acid produced during lactic acid fermentation is reported to be more toxic than alcohol produced during alcoholic fermentation. Thus, in the genotypes Ayali, Thilarani, *S.malabaricum* and SVPR1 which showed lower LDH activity and higher ADH and PDC activity, the damage in root tissue was less as indicated by higher root number and root dry weight. This result is in agreement with the findings of Wei *et al*. (2013) in sesame.

5.2.4 Anatomical study

Aerenchyma formation is a major physiological and morphological adaptation of plants to waterlogging or flooding conditions (Jiang *et al*., 2010). It has long been known that rapidly formed aerenchyma is critical for waterlogged plants in maintaining adequate oxygen supply and overall hypoxia tolerance (Armstrong, 1979; Evans, 2003; Armstrong and Armstrong, 2014). In the present study, more aerenchyma formation was observed in tolerant genotypes *S. malabaricum* which recorded 100 per cent survival (Plate 9). Significant formation of aerenchyma was also noticed in Ayali and Kayamkulam 1.

5.2.5 Cluster analysis

The hierarchical cluster analysis of 15 sesame genotypes with 11 morpho-physiological characters (survival, plant height, root number, root dry weight, shoot dry weight, chlorophyll content, MDA content, catalase activity, soluble protein content, NRase activity and photosynthetic rate) indicated that the genotypes can be grouped into four clusters based on Euclidean distance' using 'Average linkage method'(Table 54). Dendrogram of sesame genotypes are given in Fig 17. The intra-cluster and inter-cluster distances were calculated and shown in Table 55.

Table 54. Clustering of sesame genotypes based on morpho-physiological parameters

Cluster I	Cluster II	Cluster III	Cluster IV
TMV6	Thilatara CO 1 AT 231	GT 10 TMV 7 Kayamkulam 1 Thilak SVPR1 S. malabaricum TMV5 Thilarani Ayali	TMV 3 TMV 4

Table 55. Intra-cluster and inter-cluster distances of sesame genotypes

	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I	0.00	91.01	68.43	59.47
Cluster II	91.01	48.09	**119.40**	61.58
Cluster III	68.43	**119.40**	**49.59**	87.26
Cluster IV	59.47	61.58	87.26	44.43

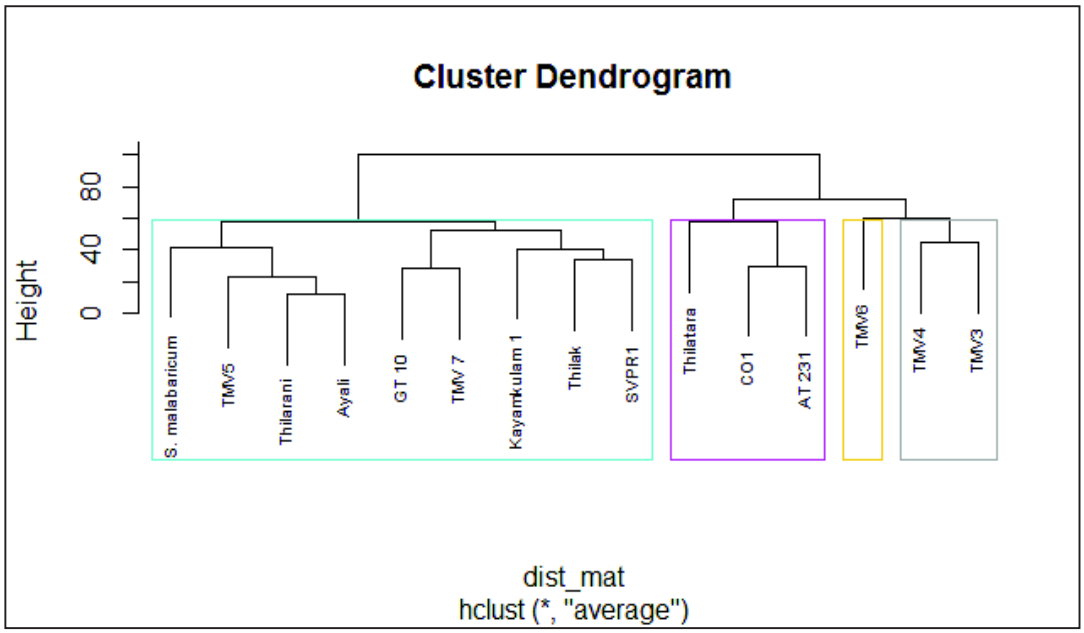


Fig.17 Dendrogram based on eleven morpho-physiological parameters of sesame genotypes under waterlogging stress

The diagonal values obtained for intra-cluster distance was found to be highest in the Cluster III. The off-diagonal values which represent the inter-cluster distance was found to be highest between the clusters II and III. Cluster II can be considered as the genotypes which are susceptible to waterlogging because these showed lowest morpho-physiological parameters under waterlogged condition, whereas cluster III can be considered as genotypes which are tolerant to waterlogging as these genotypes recorded best morpho-physiological characters under waterlogging. Hence based on the performance of genotypes in each cluster, they can be grouped as follows.

Waterlogging tolerant genotypes- GT 10, TMV 7, Kayamkulam 1, Thilak, SVPR1, *S. malabaricum*, TMV5, Thilarani, Ayali

Moderately tolerant- TMV6

Waterlogging susceptible genotypes – Thilatara, CO 1, AT 231

Moderately susceptible- TMV 3, TMV 4

5.3 EFFECT OF DROUGHT STRESS AT SEED GERMINATION STAGE IN SESAME GENOTYPES

PEG induced drought stress reduced the phenotypic expression of the seedling traits such as germination, shoot length and root length. This reduction negatively affected the vigour of all sesame genotypes. These results are in consistent with those of other works that have also reported a reduction in all the germination parameters under PEG induced drought stress in the crop (Boureima *et al*., 2011, Vignesh *et al*., 2018; Bahrami *et al*., 2012; Bijeh, 2012). In the current study, seeds were soaked for one day in water prior to stress induction, and gradually increased the concentration of PEG. This will help to screen genotypes based on their cellular tolerance capacity as it results in induction of stress responsive genes in tolerant genotypes (Bangi *et al*., 2020).

Although stress negatively affected all the genotypes under study, there was substantial difference in germination potential between the different genotypes. In

this study, overnight water soaking was done before stress induction, so the parameter which was given more importance was the percentage of normal seedling rather than the total germination percentage. Based on the number of normal seedling produced, Kayamkulam 1 (66.67 %), TMV3 (66.08 %), Ayali (65.00), Thilatara (62.22 %) and TMV6 (56.23%) were regarded as tolerant (Fig. 18).

Similarly the number of abnormal seedlings is also an important parameter that demonstrate the lower capacity of sesame to tolerate drought. Seven genotypes Ayali, Kayamkula 1, Thilatara, Thilarani, TMV3, *Sesame malabaricum* and Thilak recorded less than 30 per cent abnormal seedlings (Fig. 18). The percentage of normal and abnormal seedlings in sesame genotypes are given in Fig. 18.

Following initiation of germination, the post-germinative elongation of root and shoot tissues is regarded as an essential parameter for evaluating drought tolerance at early growth stages (Idrissi *et al*., 2015) with shoot tissues being more susceptible to water deficiency (Okcu *et al*., 2005; Abd Allah *et al*., 2010). All the 15 genotypes tested showed a reduction in shoot elongation under stress condition (Table 39). This indicates that the decrease in osmotic potential might have led to drastic inhibition of shoot tissue elongation (Kalefetoglu *et al*., 2009; Yucel *et al*., 2010). *Sesamum malabaricum* recorded least reduction in shoot length (30.35%) whereas CO1 recorded highest reduction (71.21%) (Table 39).

Root length at seedling stage provides a fair estimate about the root growth in field (Ali *et al*., 2011; Rajendran *et al*. 2011), as it is the place where plants first encounter waterstress, it is likely that roots may be able to sense and respond to the stress condition (Xiong *et al*. 2006; Khodarahmpour, 2011). The genotypes TMV3, Ayali, Kayamkulam 1 and Thilatara recorded highest root length under stress (Fig. 19). This indicates greater ability of these genotypes in penetrating the soil and to harvest water during moisture stress condition.

A reduction of all the above mentioned parameters contributed to vigour decline in tested genotypes. *S. malabaricum* (64.68 %) recorded lowest percentage reduction in seedling vigour over control, whereas CO1 (92.86%) recorded highest reduction (Fig. 20).

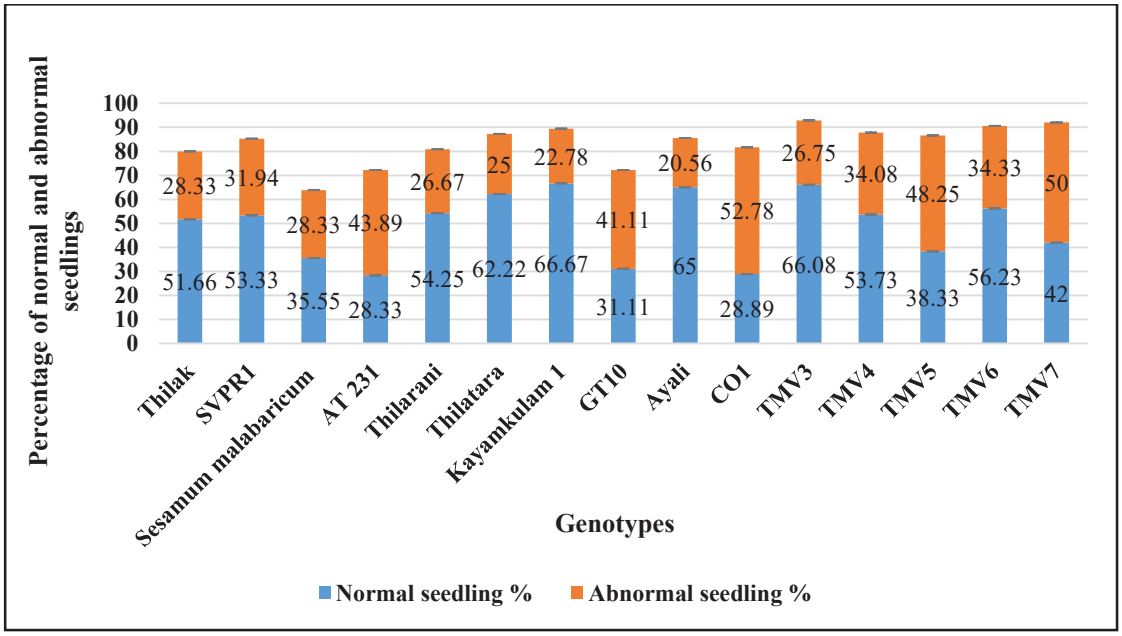


Fig. 18. Percentage of normal and abnormal seedlings under PEG induced drought condition in seame genotypes

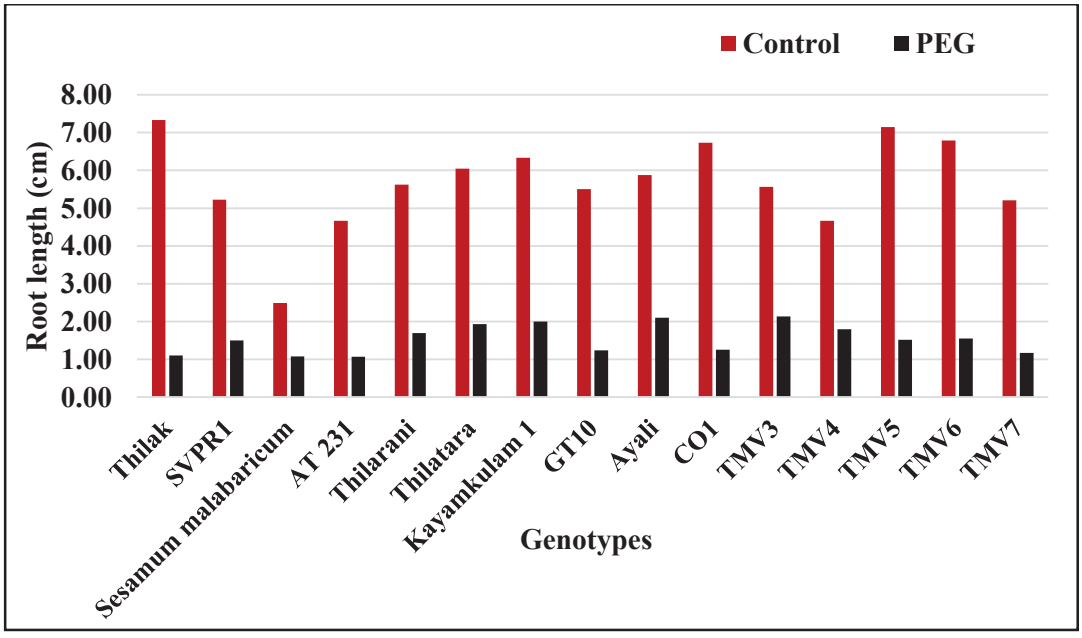


Fig 19. Root length of sesame genotypes under control and PEG induced drought stress condition

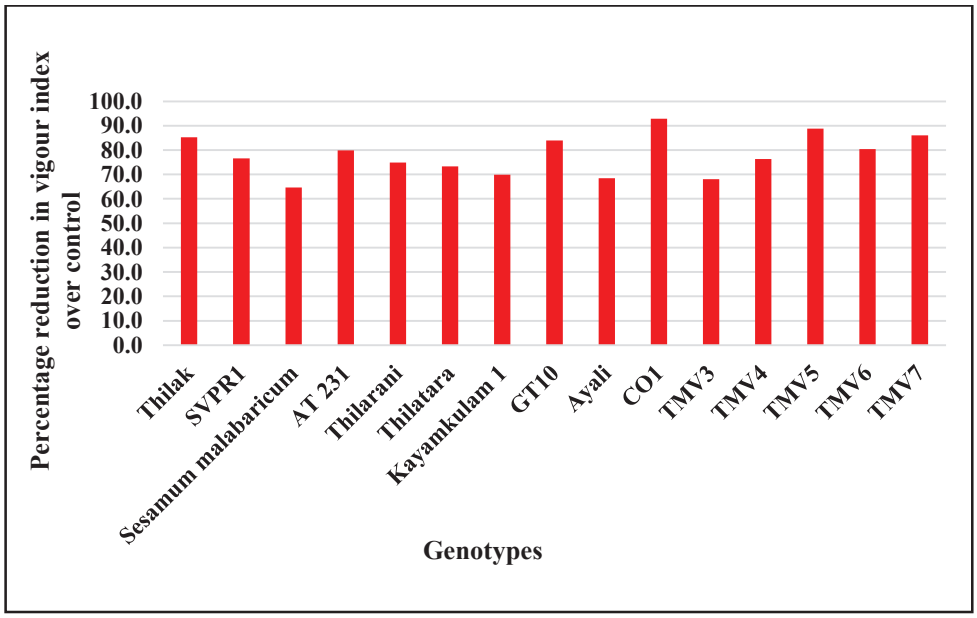


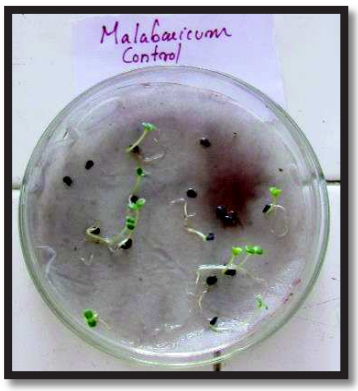
Fig. 20. Percentage reduction in vigour index of sesame genotypes under PEG induced stress

Seeds of *Sesame malabaricum* showed dormancy due to its hard seed coat as reported by Tanesaka *et al*. (2011) and Tanesaka *et al*. (2012) resulted in lower speed of germination (Table 41). Hard seedness in wild plants can be considered a favorable characteristic. It is seen that these plants remain on the same site for a long period and regenerate stands and also produce hard seeds which is important for their survival (Norman *et al*., 2002). The percentage of hard seeds increased under stress condition in *S. malabaricum*. *S.malabaricum*, a wild species can be considered as drought tolerant as it has higher number of normal seedings, lower per cent of abnormal seedlings, lower dead seedlings, least reduction in shoot length and root length and also highest stress tolerance index (35).

Among the cultivated genotypes, TMV3, Ayali, Kayamkulam 1 and Thilatara recorded lower reduction in estimated germination parameters. The genotype CO 1 was most sensitive to all the estimated parameters and can be considered as drought susceptible genotype (Plate 28). Performance of Genotypes with higher drought tolerance index is given in Plate 29.

CO 1 (control) | CO 1 (PEG)

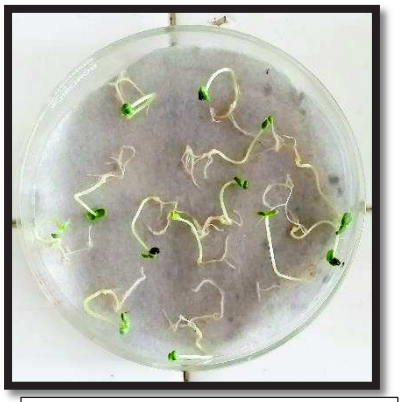
Plate 28. Performance of sesame genotype CO 1 after 7 days of germination test



S. malabaricum (control)

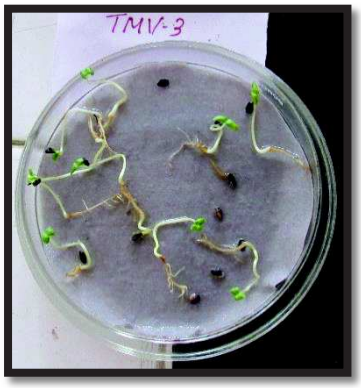
S. malabaricum (PEG)

Kayamkulam 1 (control)



Kayamkulam 1 (PEG)

TMV3 (control)



TMV3 (PEG)

Plate 29. Performance of sesame genotypes with higher drought tolerance index after 7 days of germination test

5.4 IDENTIFICATION OF SESAME GENOTYPES SHOWING SUPERIOUR TOLERANCE TO WATERLOGGING AND DROUGHT

Z-distribution analysis was adopted to identify genotypes having superior tolerance to both waterlogging and drought stress. Survival percentage under water logging was plotted on the X axis and percentage reduction of normal seedling under PEG screening was plotted on the Y axis (Fig.21). The genotypes tolerant to waterlogging and susceptible to drought fell in the quadrant I. Genotypes susceptible to both drought and waterlogging stress fell in quadrant II (-X, +Y). In quadrant III genotypes tolerant to drought and susceptible to waterlogging were observed. Genotypes tolerant to both drought and waterlogging fell in the quadrant IV, as they had higher (positive) survival and least reduction of normal seedling. Hence a positive Z score for X axis and negative Z-score for Y axis were the preferred values. The result was the same when other parameters like survival per cent under waterlogging (X axis) and reduction in vigour index under PEG (Y axis) (Fig. 22) and Change in root number under waterlogging (X axix) and change in root length under PEG (Y axis) (Fig. 23) were plotted in scatter diagrams.

Based on the response on genotypes to the different stress situations as obtained from the three scatter diagrams it was possible to classify the sesame genotypes as tolerant, moderately tolerant, moderately susceptible and susceptible genotypes to both stress situations as given in Table 56.

Table 56. Classification of sesame genotypes based on responses to waterlogging and PEG induced drought stress

Response to stress	**Genotypes**
Tolerant to both stress	*S. malabaricum*
Moderately tolerant to both stress	Ayali, Thilarani, Kayamkulam1
Moderately susceptible to both stress	AT231
Susceptible to both stress	CO 1
Waterlogging tolerant and drought susceptible	TMV5, TMV 7
Drought tolerant and waterlogging susceptible	Thilatara

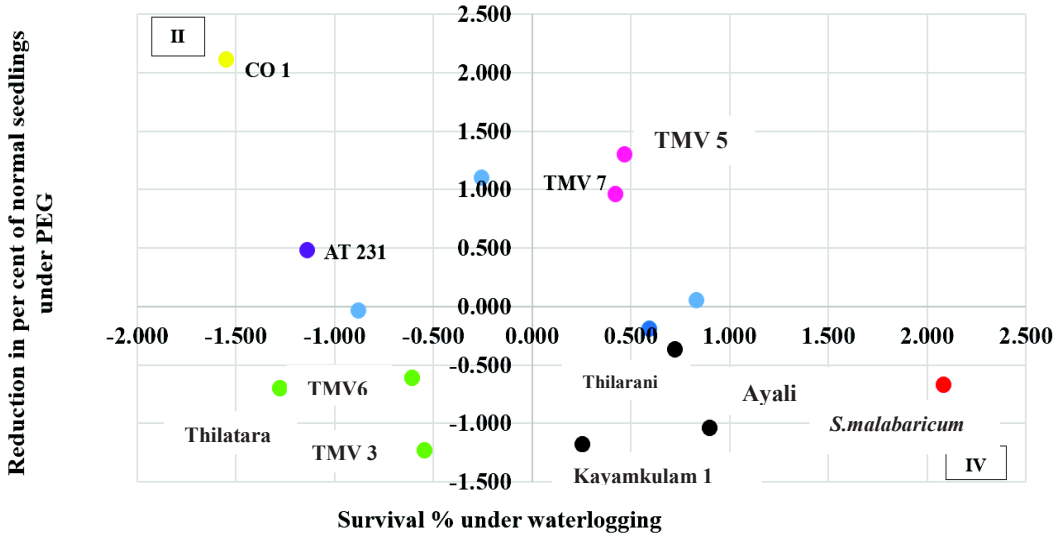


Fig 21. Z scatter diagram depicting the comparative performance of sesame genotypes under waterlogging and drought in terms of survival percentage (waterlogging) and reduction in percentage of normal seedlings (PEG). Red colour indicate tolerant *S. malabaricum*, Black colour indicates moderately tolerant Ayali, Thilarani and Kayamkulam 1. Yellow colour indicates susceptible CO 1 and violet colour indicates moderately susceptible AT231. Rose colour indicates waterlogging tolerant but drought susceptible TMV 5, TMV7. Green colour indicates drought tolerant but waterlogging susceptible Thilatara, TMV6 and TMV3.

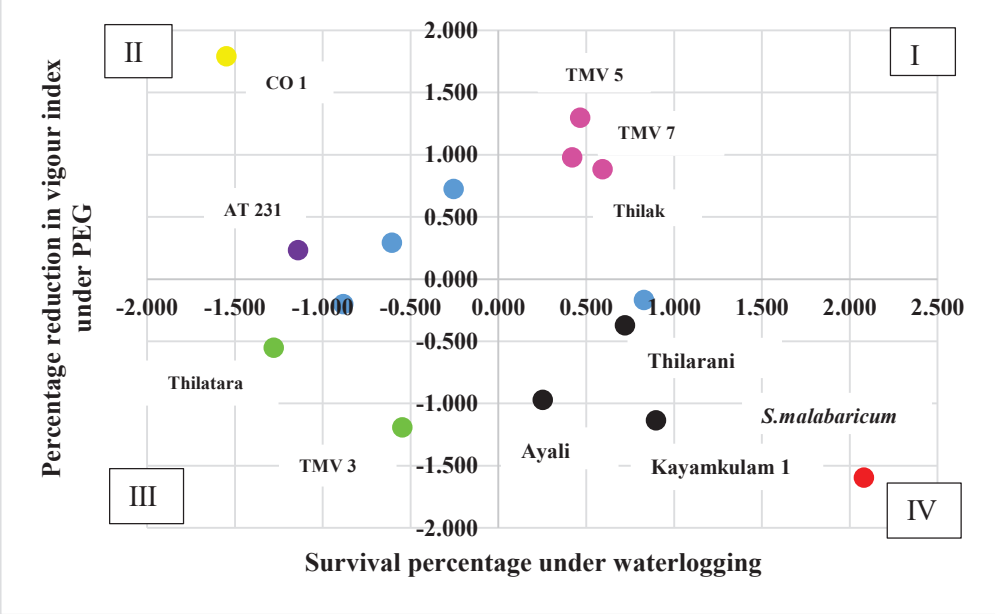


Fig 22. Z scatter diagram depicting the comparative performance of sesame genotypes under waterlogging and drought in terms of survival percentage (waterlogging) and percentage reduction in vigour index (PEG). Red colour indicate tolerant *S. malabaricum*, Black colour indicates moderately tolerant Ayali, Thilarani and Kayamkulam 1 and SVPR 1. Yellow colour indicates susceptible CO 1 and violet colour indicates moderately susceptible AT231. Rose colour indicates waterlogging tolerant but drought susceptible TMV 5, TMV7, Thilak. Green colour indicates drought tolerant but waterlogging susceptible Thilatara, TMV3.

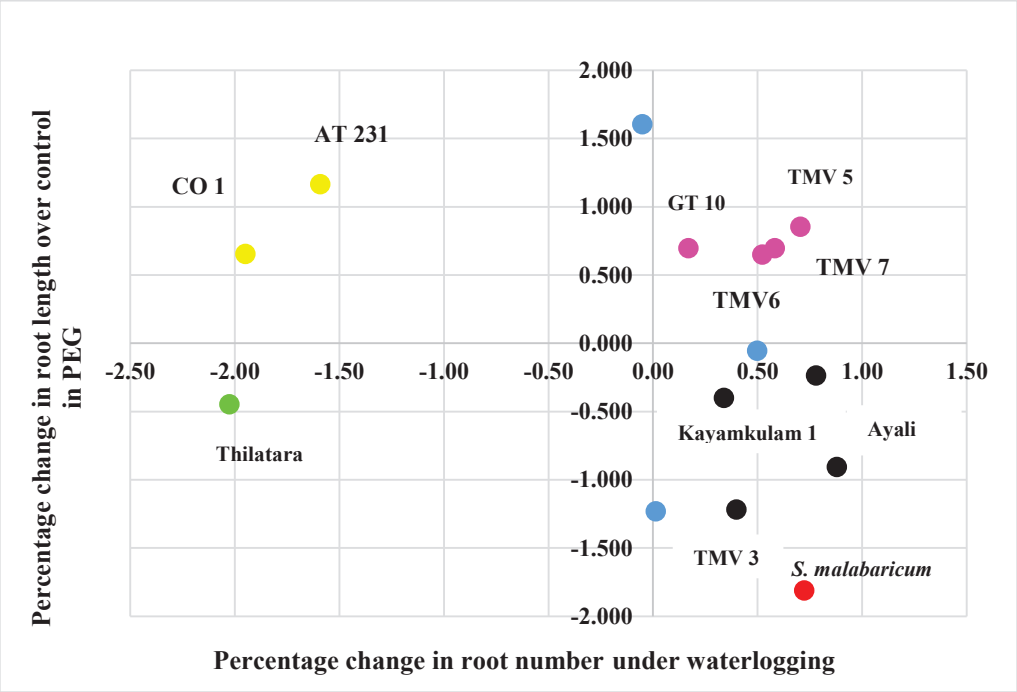


Fig 23. Z scatter diagram depicting the comparative performance of sesame genotypes under waterlogging and drought in terms of change in root number under waterlogging and change in root length under PEG. Red colour indicate tolerant *S. malabaricum*. Black colour indicates moderately tolerant, TMV3, Ayali, Kayamkulam 1 and Thilarani. Yellow colour indicates susceptible CO 1 and AT231. Rose colour indicates waterlogging tolerant but drought susceptible TMV 5, TMV7, TMV6 and GT 10. Green colour indicates drought tolerant but waterlogging susceptible Thilatara.

5.5. MOLECULAR CHARACTERIZATION OF SESAME GENOTYPES

PCR amplification of SSR markers revealed there was no polymorphism in the selected sesame genotypes (Plate 12, 13). Moreover, SSR marker ZM 428, which is tightly linked to QTL for waterlogging tolerance has reported with 520-540 bp (Zhang *et al*., 2014), whereas the allele amplified in the selected sesame genotypes were 500 bp. These SSR markers were found to be not associated with the waterlogging tolerance in the selected sesame genotypes. This suggest that different mechanism of waterlogging tolerance in the selected tolerant genotype *S. malabaricum* compared to the Chinese sesame cultivar Zhongzhi No.13 having QTL for submergence tolerance and limked SSR markers as reported by Zhang *et al*. (2014).

Phosphoenol pyruvate carboxylase in seeds of many oil plants has been reported (Sangwan *et al*., 1992; Aivalakis *et al*. 2004; Sebei *et al.* 2006). According to Sebei *et al*. (2006), *Phosphoenol pyruvate carboxylase* is involved in fatty acid and triacylglycerol biosynthesis during seed maturation in oilseed crop rapeseed. So, its activity is important for the oilseed crop sesame also. The C3 *Phosphoenol pyruvate carboxylase* was supposed to be related to the response to environmental stress (Sanchez *et al*. 2006). Comparison of *Phosphoenol pyruvate carboxylase* from the moderately tolerant genotypes and susceptible genotype, Thilatara recorded SNPs in the translated sequence of susceptible genotype that can result in truncation in amino acid sequence, change in amino acid sequence and insertion of stop codon (Fig. 5). These changes in the amino acid especially insertion of stop codon can affect the synthesis of the enzyme. RNA Interference-based suppression of *Phosphoenolpyruvate Carboxylase* results in susceptibility of rapeseed to osmotic stress interms of high lipid peroxidation (Chen *et al*., 2010). Stomatal opening was delayed in *Phosphoenol pyruvate carboxylase* antisense potato plants, but was accelerated in plants overexpressing the gene compared to the controls (Gehlen *et al*., 1996).

Xyloglucan endotransglycosylase carboxylase is a putative cell wall loosening enzyme is reported to be triggered by ethylene accumulation under

hypoxia (Saab and Sachs, 1996). The induction of the gene expression is mediated by ethylene and contributes to aerenchyma formation in roots *via* cell wall dissolution (Tong *et al*., 2021). Comparison of *Xyloglucan endotransglycosylase carboxylase* from tolerant *S. malabaricum* and susceptible CO 1 indicates SNPs in the susceptible genotype that can cause truncation of amino acid sequence affecting the protein synthesis (Fig. 6). This can be justified by the absence of aerenchyma in susceptible CO1 (Plate 10) and more aerenchyma in tolerant *S. malabaricum* (Plate 9). An upregulated transcript level of the gene expression was detected in barley (Luan *et al*., 2018) and maize roots (Saab and Sachs, 1996) under waterlogged condition. The involvement of the genes in imparting waterlogging tolerance in the selected sesame genotypes can be confirmed only after validation.

5.6 WATERPROOFING SESAME

5.6.1 ROS production and scavenging affected by ameliorants

A balance between ROS production and ROS scavenging activity decides the fate of cell under waterlogging stress (Hsu *et al*. 2000; Kato *et al*. 2001). Catalase is an antioxidant enzyme for the detoxification of ROS thereby minimizing the MDA production. In this study it was observed that highest MDA content (Fig. 24) and lowest catalase activity (Fig. 25) was recorded in control 1, which implies an unbalanced system of ROS production and scavenging. It is clear from Table 51 that all the ameliorants used in the experiment increased the catalase enzyme activity, which resulted in scavenging of ROS, thereby reducing MDA production. This is in conformity with the results obtained with salicylic acid in Peony by Zhu *et al*. (2020) and with KNO_3, $Ca(NO_3)_2$, urea and tricyclazole by Habibzadeh *et al*. (2013) in Canola under waterlogged conditions.

5.6.2 Nitrate reductase enzyme activity, soluble protein content and chlorophyll content as affected by ameliorants

Under waterlogged condition nitrate availability to the plants is a major problem. This is evident in the reduced nitate reductase acivity of control 1 plants (table 51). According to Lopez-Cantarero *et al*. (1997), increase in activity of nitrate

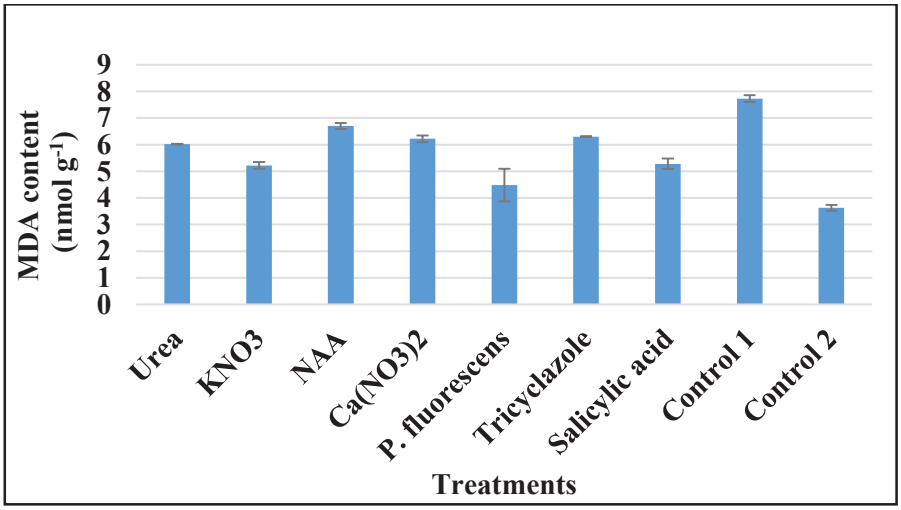


Fig 24. Effect of treatments on MDA content of sesame var. Thilak waterlogged at vegetative stage

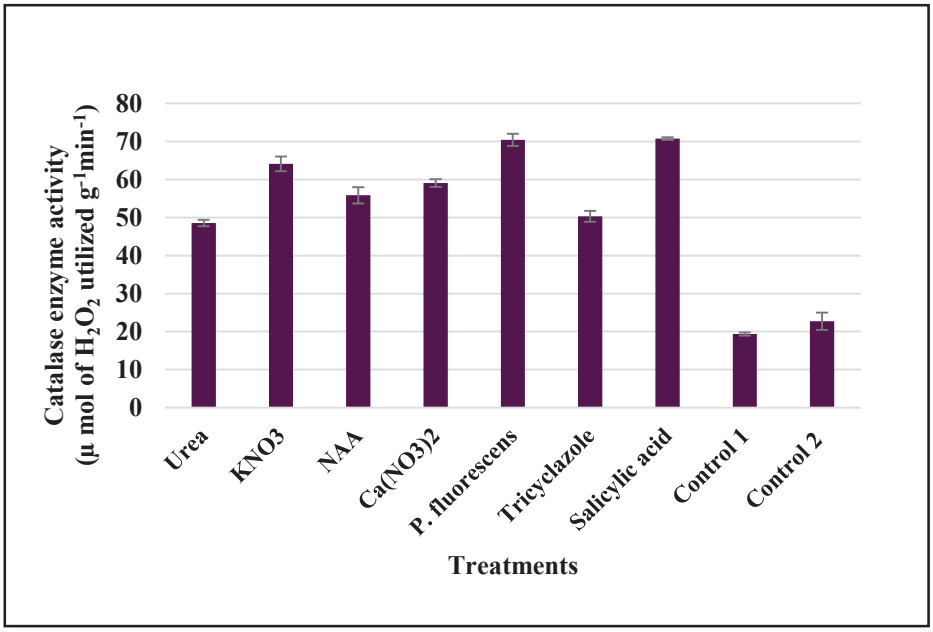


Fig 25. Effect of treatments on Catalase enzyme activity of sesame var. Thilak waterlogged at vegetative stage

reductase leads to a corresponding increase in the potential for nitrate reduction and confers a greater capacity for amino acid synthesis, protein synthesis, or total N assimilation. Application of NO_3^- as KNO_3 and $Ca(NO_3)_2$ increased the nitrate reductase activity in sesame plants (Table 51). Enhanced nitrate reductase activity in waterlogged sugarcane was reported with the foliar application of KNO_3, urea and $Ca(NO_3)_2$ (Jain *et al*., 2015). According to Abd-El-Baki *et al.* (2000) and Ghasemzadeh and Jaafar (2013), salicylic acid has a protective role in nitate reductase activity by increasing the liberation of nitrate from vacuoles upon salicylic acid application which favour the induction of nitrate reductase activity. Sharma (2015) reported an increase in activity of nitrate reductase by the inoculation of *Withania somnifera* with *Pseudomonas flouresence*. Waterlogging also reduced the soluble protein content in control 1 plants. Treatment with *P. fluorescens* showed increase in soluble protein content compared to control 1 plants which may be due to synthesis of stress related proteins (Fig 26).

Waterlogging resulted in decline in chlorophyll content in sesame var. Thilak (Table 51). All the ameliorant sprays improved the chlorophyll content as shown in Fig 27. Inhibition of nitrogen uptake is reported to be associated with plants growing in waterlogged soil (Jain *et al*., 2015). Foliar application of fertilizers were reported to be effective than soil application under waterlogging. Foliar spray of nitrogenous compounds *viz*., KNO_3 and $Ca(NO_3)_2$ to waterlogged sesame plants thus alleviated the deficiency of nitrogen thereby reduced chlorophyll degradation (Table 51). Foliar application and seed treatment of *Pseudomonas* has been reported to enhance nitrogen uptake in rice (Elekhtyar, 2015). Reduction in chlorophyll degradation upon application of salicylic acid was reported by Zhu *et al*. (2020) in waterlogged Tree peony (*Paeonia suffruticosa* Andr.). Enhanced catalase activity upon treatment application also should have reduced the chlorophyll destruction under waterlogging.

5.6.3 Effect of ameliorants on physiological parameters

One of the first plant responses to waterlogging is the reduction in stomatal conductance (Folzer *et al*., 2006) as observed in waterlogged sesame plants (control 1) (Table 50). Decreased transpiration due to reduced hydraulic conductivity attributed by the hampered root permeability under low oxygen level resulted in stomatal closure. Reduced chlorophyll content, stomatal closure and oxidative stress resulted in decline in photosynthetic rate under waterlogging (fig. 28). Application of ameliorants especially KNO_3, SA and *P. fluorescens* had a marked improvement in stomatal conductance (Table 50) and photosynthesis (Fig 28). Role of potassium in stomatal regulation, osmoregulation, photosynthetic pigments and photosynthetic capacity *via* its effects on the activities of several photosynthetic enzymes have been established (Ashraf *et al*. 2011;Taiz and Zeiger 2010). Increase in photosynthetic parameters with the foliar application of potassium have been reported by Ashraf, (2011). Plants treated with salicylic acid increased the stomatal conductance (Table 50). This proves that the application of salicylic acid can reverse the stomatal closure of sesame seedlings under flooding and improve the photosynthetic rate (Fig. 28). This result was in agreement with the finding of Zhu *et al*. (2020) in peony cultivars. According to Wang *et al.* (2010b) salicylic acid is also correlated with RUBISCO activation under abiotic stress condition.

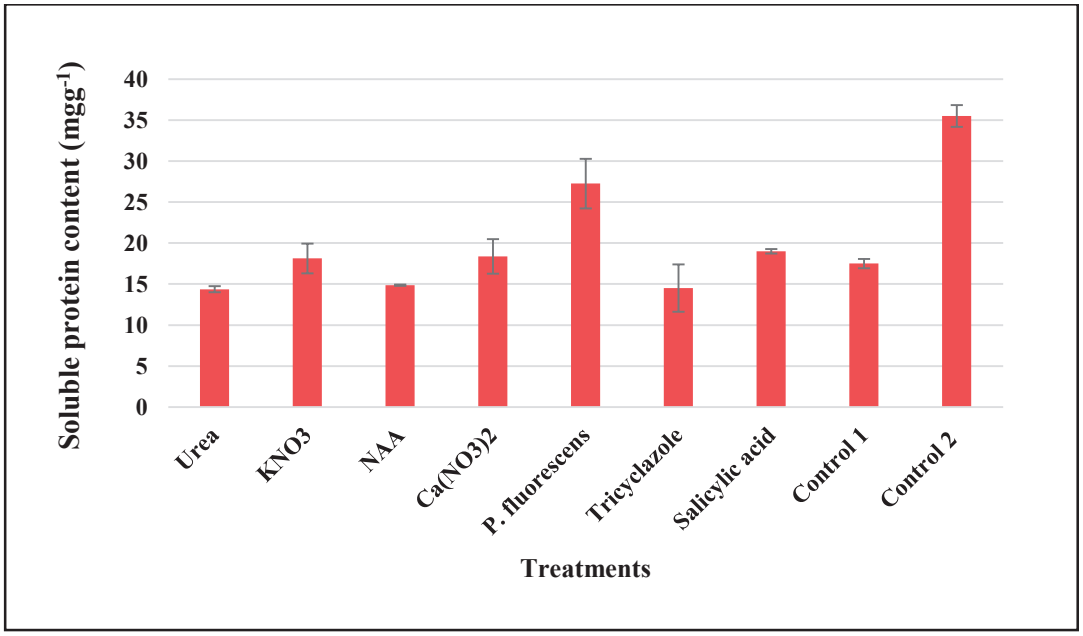


Fig 26. Effect of treatments on soluble protein content of sesame var. Thilak waterlogged at vegetative stage

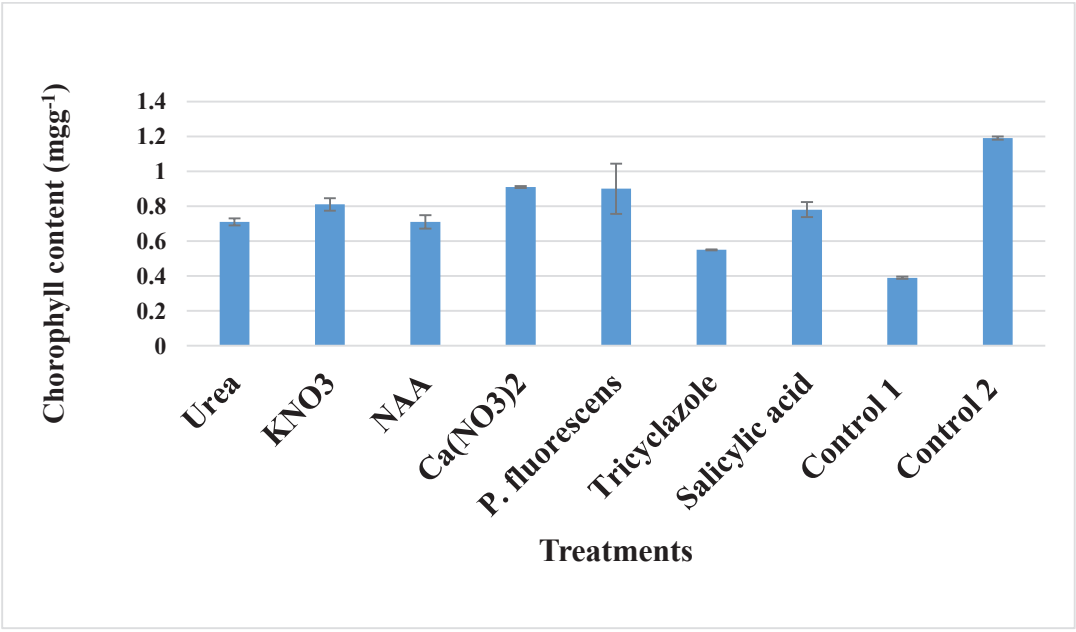


Fig 27. Effect of treatments on chlorophyll content of sesame var. Thilak waterlogged at vegetative stage

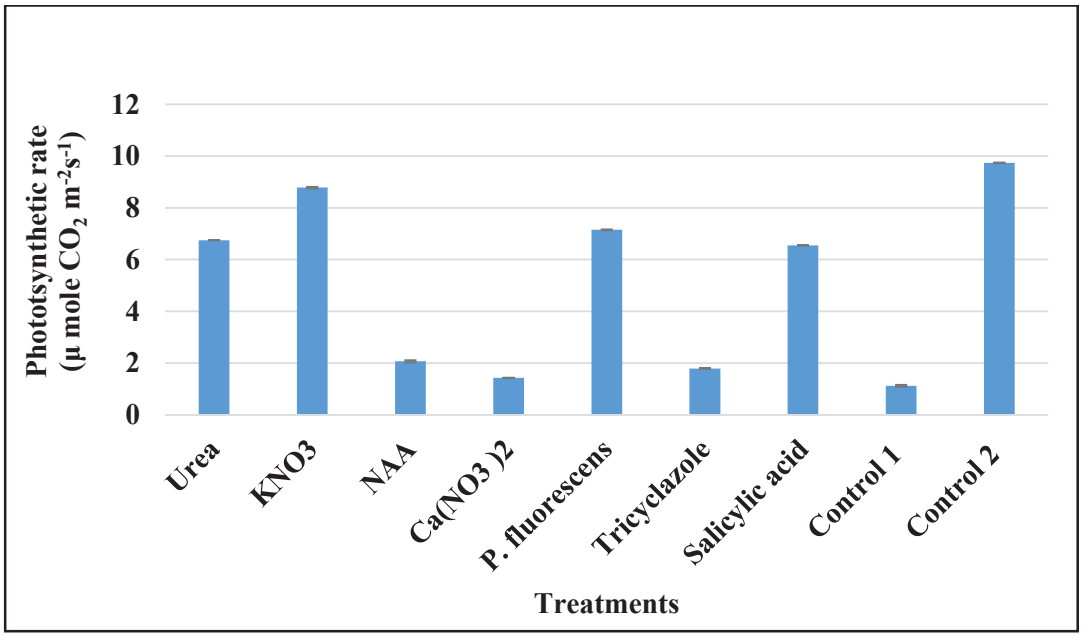


Fig 28. Effect of treatments on photosynthetic rate of sesame var. Thilak waterlogged at vegetative stage

5.6.4 Effect of ameliorants on adventitious root production

Ability of plant roots to take up and transport mineral nutrients is significantly impaired under waterlogging (Ashraf and Rehman, 1999; Malik *et al*. 2001), making nutrient delivery to the shoot inefficient. Formation of adventitious roots, located close to the surface provids better access to oxygen (Marschner 1995; Garthwaite *et al.,* 2003; Visser and Voesenek, 2005). Increase in root number with the application of salicylic acid, tricyclazole, *Pseudomonas* and KNO_3 is an indication of adventitious root production enhanced by these treatments (Table 49).

5.6.5 Effect of ameliorants on improving morphological parameters

Waterlogging resulted in overall growth reduction in terms of reduced plant height (Table 48), shoot (Table 48) and root dry weight (Table 49). This may be due to the unavailability of essential nutrients, as waterlogging is also known to inhibit the uptake of some important nutrients such as N, P, K, Ca^{2+}, $Mg^{2+}$and Fe^{2+} by crop plants (Gutierrez Boem *et al*., 1996). Root growth noticed under waterlogging might have also contributed to reduction in plant growth. Foliar application of KNO_3, urea, $CaNO_3$ partially reduced growth inhibitory effect of waterlogging by foliar supply of essential nutrients. Triazoles have both fungitoxic and plant-growth regulatory effects. They induce stress resistance by altering the hormone levels by inhibition of gibberellin synthesis, reducing ethylene evolution and increasing cytokinin levels (Kamoutsis and Chronopoulou-Sereli, 1999), increased chlorophyll content, enlarged chloroplasts, thickening leaf tissue (Watson and Himelick, 2004), enhancing both non-enzymatic and enzymatic antioxidant potentials (Kishorekumar *et al*., 2008). *Psuedomonas flouresence* was reported to enhance growth of plants by synthesis of substances such hormones e.g. auxin *i.e*. indole acetic acid (IAA), abscisic acid (ABA), gibberellic acid and cytokinins, nitrogen fixation, solubilization of phosphorus and mineralization of other nutrients, sequestering of iron cytokinins, gibberellins, and facilitation of nutrient uptake (Elekhtyar, 2015). Salicylic acid was reported to have a role in ion uptake, transport (Glass, 1975) and growth promotion (Arfan *et al*., 2007). Potassium supplementation improved nutrient uptake of waterlogged cotton plants resulted in significantly higher accumulation of K^+, Ca^{2+},

N, Mn^{2+} and Fe^{2+} than those plants that were not supplied with K (Ashraf *et al*., 2011). The application of NAA resulted in poor performance because the application dose was not appropriate for the initial stage of the crop as evident from the leaf scorching associated with spraying.

Improvement in biochemical and photosynthetic parameters resulted in improvement in survival of plants. This was true for treatments KNO_3, *P. flourescens* and Salicylic acid, Tricyclazole and Urea as seen in Fig 29. Morphological response of waterlogging in sesame var. Thilak with superior treatments is given in Plate 30.

5.6.6 Effect of ameliorants on improving sesame yield

All the stress related growth parameter in waterlogged sesame plants showed response mostly to the application of *Pseudomonas fluorescens*, salicylic acid and KNO_3. Improvement in physiological and biochemical response along with growth promotion with these treatments resulted in enhanced yield compared to the untreated waterlogged plants (control 1) (Fig. 30). Application of *Pseudomonas*, KNO_3 and salicylic acid before waterlogging stress resulted in enhanced production of branches (Table 53), capsules per plant (Table 53) and seeds per capsules (Table 53) compared to untreated control (control 1). Control 1recorded 4.66 g yield per plant which resulted in 23 per cent yield reduction compared to control 2. Sarkar *et al*. (2016) reported 51.67 per cent and 58.24 per cent yield decline in two sesame varieties *viz*., BARI Til 2 and BARI Til 3 under 36 hrs of continuous waterlogging. Reduction in yield was minimized by application of *P. fluorescens*, KNO_3 and salicylic acid. These treatments recorded 16.52, 13.73 and 7.93 per cent improvement in yield respectively as compared to control 1 (Fig. 31). These results indicate that by application of these ameliorants 2 days before waterlogging of sesame at vegetative stage (20 DAS), 7.85 to 17.14 per cent improvement in yield per hectare can be obtained in the variety Thilak.

Plate 30 A. *P. flourescens* treatment

Plate 30 B. KNO_3 treatment

Plate 30 C. Salicylic acid treatment

Plate 30 D. Waterlogged control

Plate 30. Morphological response of sesame var. Thilak treated with *P. flourescens* (A), KNO_3 (B) Salicylic acid (C) and Non-treated waterlogged control (D)

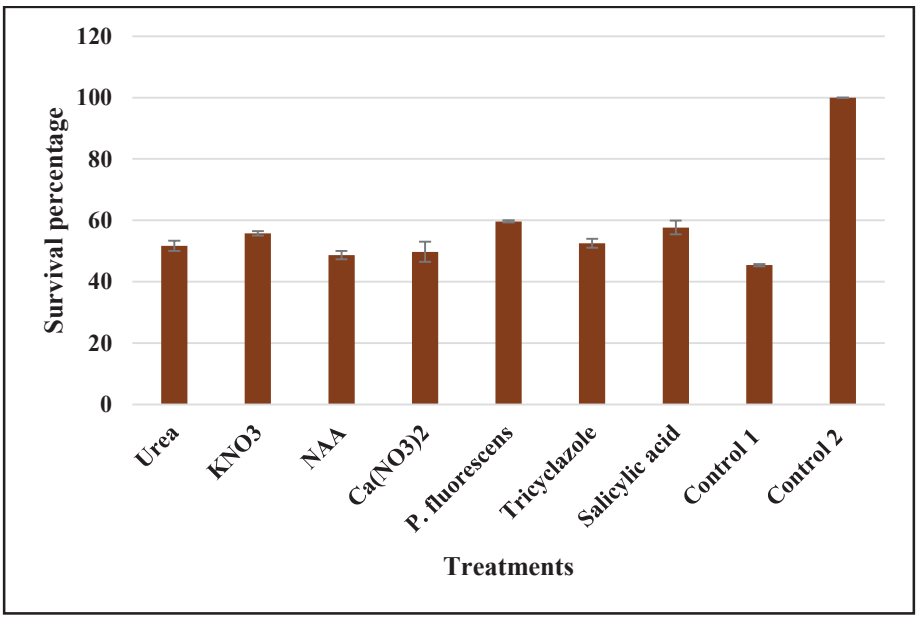


Fig 29. Effect of treatments on survival percentage of sesame var. Thilak waterlogged at vegetative stage

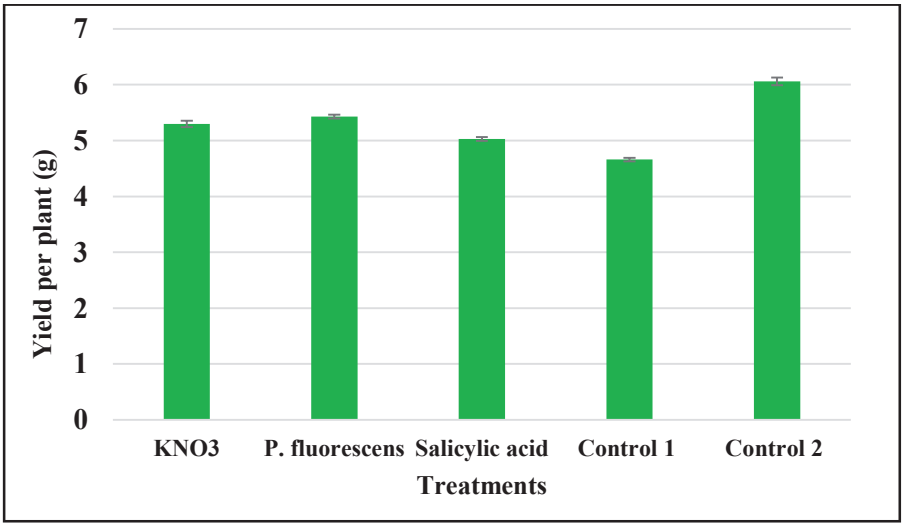


Fig 30. Effect of ameliorants on yield per plant of sesame var. Thilak

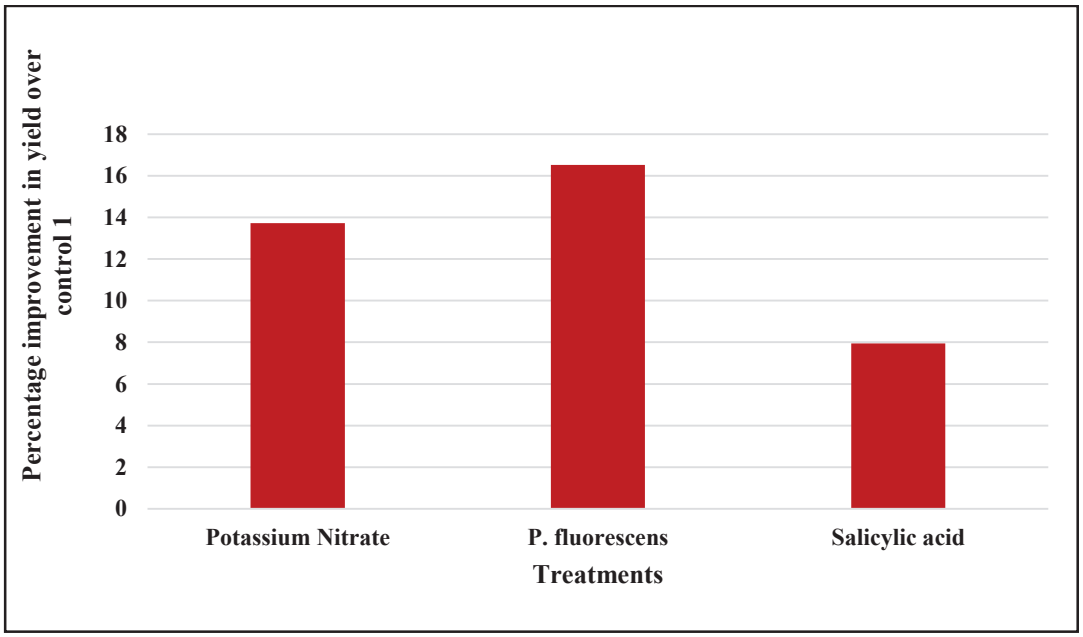


Fig 31. Percentage improvement in yield of sesame var. Thilak with ameliorant application over non treated waterlogged control (control 1)

Summary

6. SUMMARY

The project entitled "evaluation of sesame genotypes for tolerance to waterlogging and development of mitigation strategies" focused on identifying the constraints in sesame production, screening of sesame genotypes for tolerance to water logging and drought stress, molecular characterization of genotypes with stress related markers and to identify suitable amelioratives for making waterproofing sesame crop.

Salient findings of the study are

6.1. Farmer survey

- High labour cost is identified as the major problem faced by sesame growers in Kerala. Among the abiotic stresses, waterlogging and drought were reported to be the top constraints.

6.2. Screening sesame genotypes for tolerance to waterlogging

- Screening of sesame genotypes for tolerance to waterlogging at vegetative and reproductive stage revealed that initial stage was more susceptible to waterlogging.
- Among the genotypes under study, *S. malabaricum* possessed 100 per cent survival after 3 days of waterlogging. Among the cultivated genotypes Ayali, Thilarani and SVPR 1 recorded higher survival.
- The sesame genotype CO 1 recorded lowest survival under both stages of waterlogging
- Production of adventitious roots close to the soil surface is an important adaptive character under waterlogging. Tolerant genotypes produced more roots while the root growth of susceptible genotypes were negatively affected.
- MDA production was found to be a fair estimate of waterlogging tolerance in sesame genotypes as the tolerant genotypes (*S.malabaricum*, Ayali, Kayamkulam 1 and Thilarani) recorded lowest and susceptible genotypes (Thilatara and CO1) recorded highest MDA content.

- Chlorophyll content, nitrate reductase enzyme activity and soluble protein content was reduced under waterlogged condition. The reduction was more pronounced in susceptible genotypes.
- Estimation of anaerobic enzyme activity of tolerant and susceptible genotype revealed that in later LDH activity was more pronounced than ADH and PDC activity indicating the induction of lactic acid fermentation in susceptible genotypes.
- Aerenchyma formation was observed under waterlogging in tolerant genotypes. *S. malabaricum* produced more erenchyma.
- Cluster analysis of the 15 genotypes based on morpho-physiological parameters revealed that the genotypes *S. malabaricum*, GT 10, TMV 7, Kayamkulam 1, Thilak, SVPR1, TMV5, Thilarani and Ayali were grouped under one cluster. These varieties were recorded to be more tolerant to waterlogging whereas CO1, Thilatara and AT231 formed another cluster which is grouped into more waterlogging susceptible
- The study revealed the morphophysiolological mechanism of tolerance to waterlogging in tolerant sesame genotypes (Plate 31). Incresed root number, aerenchyma formation and upregulation of fermentative glycolytic enzymes are the root adaptation observed. In the leaves increased antioxidant system (catalase) reduced the rate of lipid peroxidation (MDA content), thereby balancing ROS generation and detoxification. The antioxidant enzymes can also protect nitrate reductase, soluble protein and chlorophyll from its degradation thus improving photosynthetic parameters. These changes finally contributes to increased survival under waterlogging (Plate 31).

6.3 Screening for PEG induced drought stress at seed germination stage

- The seedling growth parameters under study was (Per cent of normal seedling, shoot length, root length, vigour index) found to be affected negatively under PEG induced drought stress.
- Kayamkulam 1 (66.67 %), TMV3 (66.08 %), Ayali (65.00), Thilatara (62.22 %) and TMV6 (56.23%) produced higher per cent of normal seedlings under PEG induced stress condition.

- Under PEG induced drought stress condition, Ayali, Kayamkulam 1, Thilatara, Thilarani, TMV3, *Sesame malabaricum*, Thilak recorded less than 30 per cent abnormal seedlings whereas CO1 (52.78 %), TMV 7 (50.00%), TMV5 (48.25 %), AT231 (43.89 %) and GT10 (41.11 %) recorded higher per cent of abnormal seedlings
- Root length is an important indicator of drought tolerance in plants. The genotypes TMV3, Ayali, Kayamkulam 1 and Thilatara recorded highest root length during stress condition.
- *S. malabaricum* (64.68 %) recorded lowest percentage reduction in seedling vigour over control, whereas CO1 (92.86%) recorded highest reduction.
- Seed dormancy was exhibited by *S.malabaricum* resulted in lower speed of germination. The per cent of hard seeds was increased under drought stress can be an adaptive mechanism under drought stress.
- Higher drought tolerance index was recorded in *S.malabaricum* (35), TMV3 (32), Ayali (32), Kayamkulam 1 (31) and Thilatara (27), whereas CO 1 recorded the lowest (7).

6.4 Tolerance to waterlogging and drought stress

- Z scatter analysis of important morphological parameters under waterlogging and drought stress condition revealed that the genotype *S.malabaricum* (Plate 32) has tolerance to both stress whereas CO 1 (Plate 32) was susceptible to both stress.

6.5 Molecular characterization of sesame genotypes

- SNPs were identified in the susceptible genotypes Thilatara and CO 1 for *Phosphoenolpyruvate Carboxylase* and *Xyloglucan endotransglycosylase carboxylase* respectively. So, these genes can have a role in imparting tolerance to waterlogging in the tolerant varieties. The involvent of these gene can be confirmed only after validation.

6.6 Amelioration of waterlogging stress

- Application of *P. fluorescens*, KNO_3 and salicylic acid before waterlogging imparted better tolerance to plants when they experience waterlogged condition during early vegetative stage
- The improvement in physiological and biochemical response along with growth promotion with ameliorants resulted in enhanced yield compared to the untreated waterlogged plants
- An improvement in yield of 16.52, 13.73 and 7.93 per cent was recorded from *P. fluorescens*, KNO_3 and salicylic acid compared to control 1 respectively.
- It is clear from the study that the application of *P. fluorescens*, KNO_3 and salicylic acid had profound influence on the antioxidant system (catalase) in the sesame plants that reduced its lipid peroxidation (MDA content) and protedcted chlorophyll, nitrate reductase and protein from degradation. These resulted in better growth and survival compared to non treated waterlogged plants that ultimately improved the yield attribues and yield (Fig. 32)

Future line of work

- Standardization of dose and stages of application of ameliorants for waterproofing sesame
- Adopting techniques and treatments for increasing lodging resistance in sesame under waterlogged condition
- Transcriptomic study of genes up regulated under waterlogging
- Utilization of *S. malabaricum* in crop improvement programmes

MECHANISM OF WATERLOGGING TOLERANCE IN SESAME

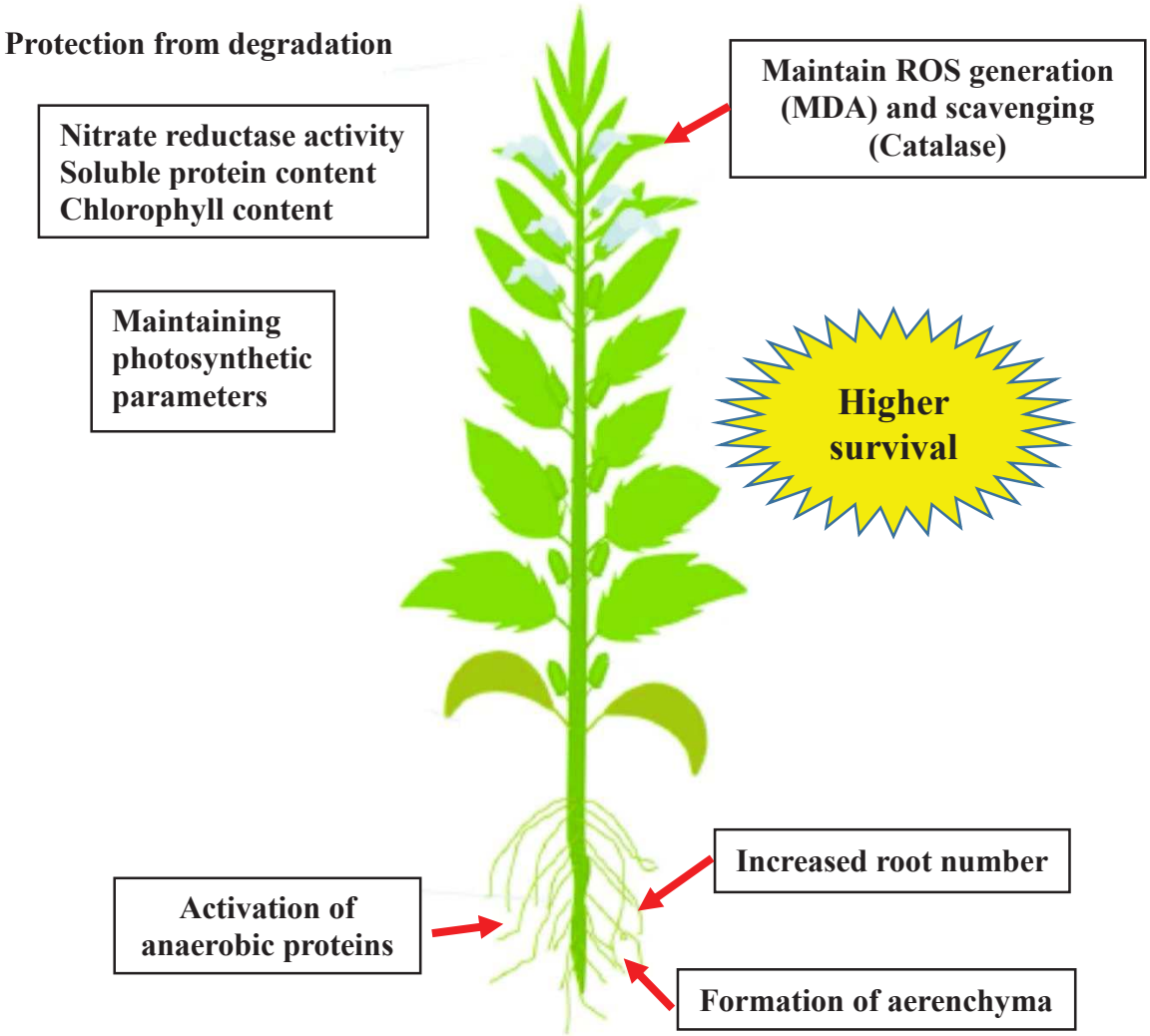


Plate 31. Diagramatic representation of mechanism of waterlogging tolerance in tolerant sesame genotypes

Sesamum malabaricum

(Tolerant to both stress)

Sesame var. CO 1

(Susceptible to both stress)

Plate 32.Tolerant and susceptible genotypes identified from the study

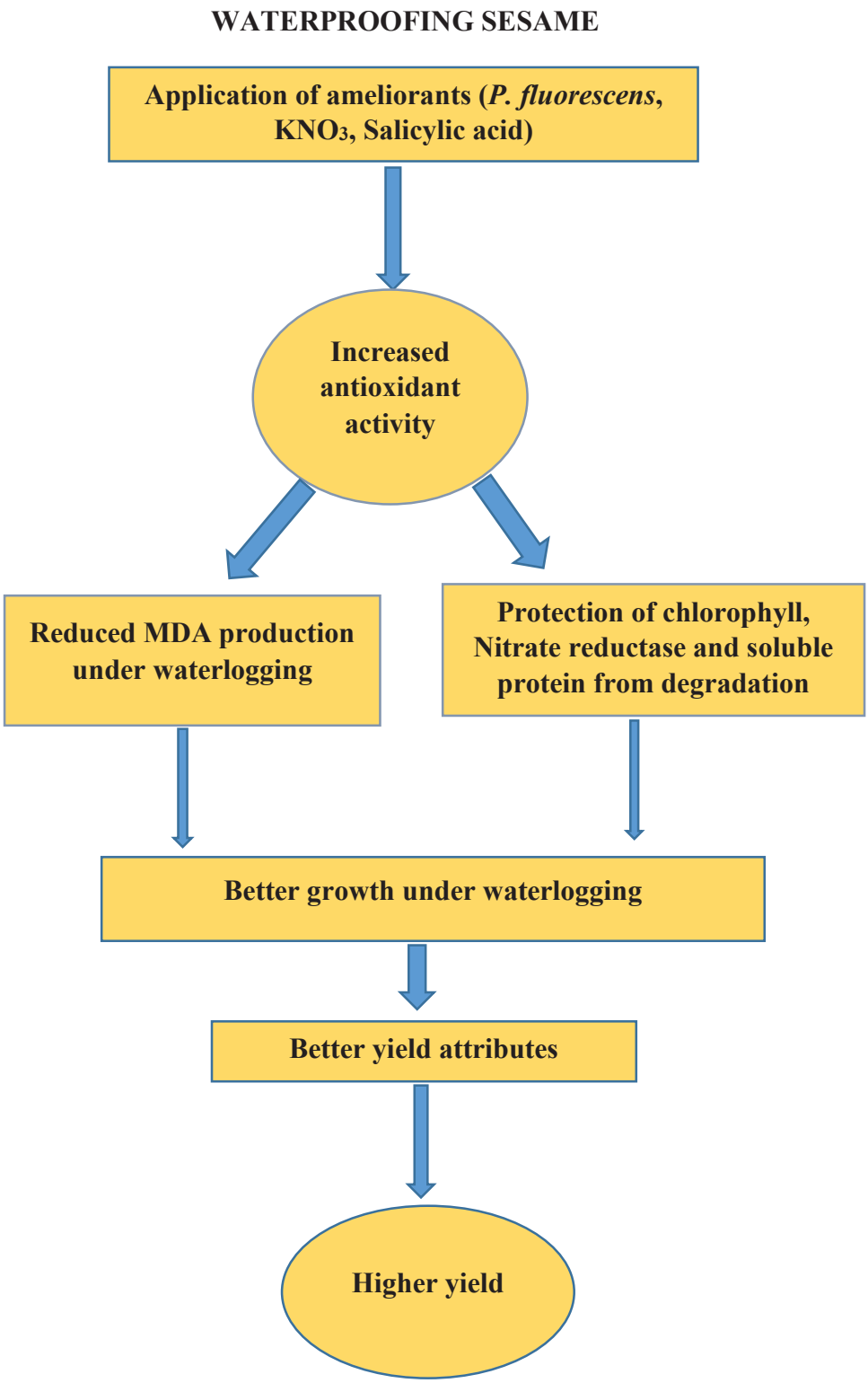


Fig. 32. Flowchart showing the waterlogging stress mitigating effect of ameliorants in sesame var. Thilak

References

References

Abd Allah, A.A., Badawy, S. A., Zayed, B.A., and ElGohary, A.A.2010. The role of root system traits in the drought tolerance of rice (*Oryza sativa* L.). *World Acad. Sci. Eng. Technol.* 68:378-1382.

Abd-ElBaki, G.K., Siefritz, F., Man, H.M., Weiner, H., Haldenhoff, R., Kaiser, W.M.2000. Nitrate reductase in *Zea mays* L. under salinity. *Plant Cell Environ.* 23:515–52.

Abdul-Baki, A. A. and Anderson, J. D., 1973. Vigour determination in soybean seed by multiple criteria. *Crop Sci*. 13:630–633.

Agius, F., Gonzalez-Lamothe, R., Caballero, J. L., Munoz-Blanco, J., Botella, M. A., and Valpuesta, V. 2002. Engineering increased vitamin C levels in plants by overexpression of a D-galacturonic acid reductase. *Nat. biotechnol.* 21:177-181.

Ahmad, S., Ahmad, R., Ashraf, M.Y., Ashraf, M., and Waraich, E.A.2009. Sunflower (*Helianthus annuus* L.) response to drought stress at germination and seedling growth Stages. *Pak. J. Bot.* 41:647–654.

Ahmed, S., Nawata, E., Hosokawa, M., Domae, Y., and Sakuratani, T. 2002. Alterations in photosynthesis and some antioxidant enzymatic activities of mungbean subjected to waterlogging. *Plant Sci*.163:117–23.

Aivalakis, G., Dimou, M., Flemetakis, E., Plati, F., Katinakis, P., and Drossopoulos, J.B.2004.Immunolocalization of carbonic anhydrase and phosphoenolpyruvate carboxylase in developing seeds of *Medicago sativa*. *Plant Physiol. Biochem*. 42: 181-186.

Al-Eqaili, S.N.M., Lahmod, N.R., and Eshkandi, O. H. 2017. Weed management in sesame field (*Sesamum indicum* L.) using wheat straw and tillage or no tillage systems. *Am. J. Agric*. *Biol. Sci*. 12(2):100-103.

Ali, A.A., Ali, T.B., and Nour, K.A.M. 2009. Antioxidants and some natural compounds applications in relation to tomato growth, yield and chemical constituents. *Ann. Agric. Sci. Moshtohor* 47(4): 469-477.

Ali, M. A., Abbas, A., Awan, S. I., Jabran, K., and Gardezi, S.D.A.2011. Correlated response of various morpho-physiological characters with grain yield in sorghum landraces at different growth phases. *J. Anim. Plant Sci*. 21(4): 671-679.

Alqudah, A., samarah, N., and Mullen, R. 2011. Drought stress effect on crop pollination, seed set, yield and quality. In: Lichtfouse, E. (ed.), *Alternative Farming Systems, Biotechnology, Drought Stress and Ecological Fertilisation*. Springer, Netherlands, pp. 193-213.

Anee, T. I., Nahar, K., Rahman, A., Mahmud, J.A., Bhuiyan, T.F., Alam, M. U, Fujita, M., and Hasanuzzaman, M. 2019. Oxidative damage and antioxidant defense in *Sesamum indicum* after different waterlogging durations. *Plants* 8(7): 196.

[Anonymous]. 2019. *Sesame seed crop report 2019 and forecasts* [on line]. Organic products India. *Available: https://www.viralspices.com/blog/sesame-seed-crop-report-2019-and-forecast* [25 Sept. 2019].

Arfan, M., Habib, A., and Ashraf, M. 2007. Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress. *J. Plant Physiol*. 164(6): 685-694.

Armstrong, W. 1979. Aeration in higher plants. In: Woolhouse, H. W. (ed.), *Advances in Botanical Research*. Academic Press, London. pp. 225–332.

Armstrong, W. and Armstrong J. 2014. Plant internal oxygen transport (diffusion and convection) and measuring and modelling oxygen gradients. In: van Dongen, J. and Licausi, T. (eds), *Low-Oxygen Stress in Plants*. Springer, Heidelberg, pp. 267–297.

Ashraf, C. M. and Abu-Shakra, S. 1978. Wheat seed germination under low temperature and moisture stress. *Agron. J.* 70:135-139.

Ashraf, M. and Mehmood, S. 1990. Effects of waterlogging on growth and some physiological parameters of four Brassica species. *Plant Soil* 121(2):203-209.

Ashraf, M. and Rehman, H.1999. Mineral nutrient status of corn in relation to nitrate and long-term waterlogging. *J. Plant Nutr*. 22:1253–1268.

Ashraf, M.A., Ahmad, M.S.A., Ashraf, M., Al-Qurainy, F., and Ashraf, M.Y.2011. Alleviation of waterlogging stress in upland cotton (*Gossypium hirsutum* L.) by exogenous application of potassium in soil and as a foliar spray. *Crop Pasture Sci*. 62:25–38.

Ashtox, T. 1948. *Technique of Breeding for Drought Resistance in Crops*. Commonwealth Bureau of Plant Breeding and Genetics, Cambridge, 34p.

Athul, V. 2016. Evaluation of sesame genotypes for tolerance to waterlogging. M. Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 128p.

Badr, A., El-Shazly, H.H., Tarawneh, R.A., and Borner, A. 2020. Screening for drought tolerance in maize (*Zea mays* L.) germplasm using germination and seedling traits under simulated drought conditions. *Plants* [e-journal] 9(5). Available: https://doi.org/10.3390/plants9050565. ISSN 2223-7747 [April 2021].

Bahrami, H., Razmjoo, J., and Jafari, A. O. 2012. Effect of drought stress on germination and seedling growth of Sesame cultivars (*Sesamum indicum* L.). *Int. J. Agric. Sci.* 2:423-428.

Bange, M., Milroy, S., and Thongbai, P. 2004. Growth and yield of cotton in response to waterlogging. *Field Crops Res*. 88, 129–142.

Bangi, S., Parvathi, M. S. and Nataraja, K. N. 2020. Development and standardization of a robust protocol to screen for cellular tolerance contributing for abiotic stress tolerance in rice. *Mysore J. Agric. Sci.*54 (1):33-40.

Baranwal, S. and Singh, B.B.2002. Effect of waterlogging on growth, chlorophylls and saccharides content in maize genotypes. *Indian J. Plant Physiol*. 7: 246-251.

Bassiri, A., Khosh-Khui, M., and Rouhani, I. 2009. The influences of simulated moisture stress conditions and osmotic substrates on germination and growth of cultivated and wild safflowers. *J. Agri. Sci*. 88: 85-100.

Bedigian, D. and Harlan, J.R. 1986. Evidence for cultivation of sesame in the ancient world. *Econ. Bot*. 40:137–154.

Beltrao, N. E., Azevedo, De. M., Nobrega, D.M.P.De., Santos, L.B.D., and Das, J. W. 1997. Changes in growth and development of annual cotton with waterlogging of the substrate in the green house. *Brazilian J. Agric. Res*. 32(4):391-398.

Bibi, A, Sadaqat, H. A., Akram, H.M., and Mohammed, M. I. 2010. Physiological markers for screening sorghum (*Sorghum bicolor*) germplasm under water stress condition. *Int. J. Agric. Biol*. 12: 451–455.

Bibi, A., Sadaqat, H.A., Tahir, M.H.N., and Akram, H.M., 2012. Screening of sorghum (*Sorghum bicolor* var Moench) for drought tolerance at seedling stage in polyethylene glycol. *J. Anim. Plant Sci*. 22(3):671-678.

Biemelt, S. U., Keetman, H.P., Mock, and Grimm, B. 2000. Expression and activity of isoenzymes of superoxide dismutase in wheat roots in response to hypoxia and anoxia. *Plant Cell Environ*. 23: 135–144.

Bijeh, K. M. H. 2012.The effect of drought stress on germination and early growth of Sesamum indicum seedling's varieties under laboratory conditions. *Int. J. Agric. Manag. Dev*. 2(4):271-275.

Bond, J.J. and Willis, W.O. 1969. Soil water evaporation: Surface residue rate and placement effects. *Soil Sci. Soc. Am. J.* 33(3):445-448.

Boureima, S., Diouf, M., Amoukou, A.I., and Van Damme, P. 2016. Screening for sources of tolerance to drought in sesame induced mutants: assessment of indirect selection criteria for seed yield. *Int. J. Pure Appl. Biosci.* 4:45–60.

Boureima, S., Eyletters, M., Diouf, M., Diop, T. A., and Damme, P. V. 2011. Sensitivity of seed germination and seedling radicle growth to drought stress in Sesame, *Sesamum indicum* L. *Res. J. Environ. Sci*. 5: 557.

Braam, J., M.L., Sistrunk, D.H., Polisensky, W., Xu, M.M., Purugganan, D.M., Antosiewicz, P., Campbell, and Johnson, K. A. 1996. Life in a changing world; TCH gene regulation of expression and responses to environmental signals. *Physiol. Plant.* 98: 909–916.

Brar, G.S. and K.L. Ahuja. 1979. Sesame: its culture, genetics, breeding and biochemistry.In: Malik, C.P. (ed.), *Annual Review of Plant Science*. Kalyani publishers, New Delhi, pp. 245-313.

Channaoui, S., El Idrissi, I.S., Mazouz, H., and Nabloussi, A. 2019. Reaction of some rapeseed (*Brassica napus* L.) genotypes to different drought stress levels during germination and seedling growth stages. *OCL-Oil seeds Fats Crops Lipids* [e-journal] 26 (1).Available: https://doi.org/10.1051/ocl/2019020.ISSN: 2272-6977 [21 March 2020].

Chen, M., Tang, Y., Zhang, J., Yang, M., and Xu, Y. 2010.RNA interference-based suppression of phospho*enol*pyruvate carboxylase results in susceptibility of rapeseed to osmotic stress. *J. Integr. Plant Biol.* 52**:**585–592.

Cheng, G., Wang, L., and Lan, H. 2016. Cloning of *PEPC-1* from a C4 halophyte *Suaeda aralocaspica* without Kranz anatomy and its recombinant enzymatic activity in responses to abiotic stresses. *Enzyme Microb. Tech*. 83**:**57–67.

Cho, S. K., Kim, J. E., Park, J. A., Eom, T. J., and Kim, W. T. 2006. Constitutive expression of abiotic stress-inducible hot pepper CaXTH3, which encodes a xyloglucan endotransglucosylase/hydrolase homolog, improves drought and salt tolerance in transgenic Arabidopsis plants. *FEBS Letters* 580: 3136–3144.

Choi, H. K., Kim, Y. J., Guh, J. O., Hwang, J. K., and Kim, H.J. 1990. Influence of waterlogging period at different growth stage on agronomic characters in sesame. *Korean J. Crop Sci*. 35(3):224-232.

Cocks, P. 2001. Ecology of herbaceous perennial legumes: a review of characteristics that may provide management options for the control of salinity and waterlogging in dryland cropping systems. *Aust. J. Agric. Res*. 52(2): 137-151.

Cong, Y., Li, Y. J., Zhou, C.J., Zou, C.S., Zhang, X.K., Liao, X., and Zhang, C.L. 2009. Effect of application of nitrogen, phosphorus and potassium fertilizers on yield in rapeseed (*Brassica napus* L.) under the waterlogging stress. *Plant Nutr. Fertil. Sci*. 15: 1122–1129.

Cousins, A. B., Baroli, I., Badger, M.R., Ivakov, A., Lea, P.J., Leegood, R.C., and von Caemmerer, S.2007.The role of phospho*enol*pyruvate carboxylase during C4 photosynthetic isotope exchange and stomatal conductance. *Plant Physiol.* 145**:**1006–1017.

De Vos, C.H.R., Schat, H., De Waal, M.A.M., Vooijs, R., and Ernst, W.H.O. 1991. Increased resistance to copper-induced damage of the root cell plasmalemma in copper tolerant *Silene cucubalus*. *Physiol. Plant*. 82: 523-528.

Dennis, E.S., Dolferus, R., Ellis, M., Rahman, M., Wu, Y., Hoeren, F.U., Grover, A., Ismond, K.P., Good, A.G., and Peacock, W.J. 2000. Molecular strategies for improving waterlogging tolerance in plants. *J. exp. Bot.* 51(342):89-97.

Dhanda, S. S., Sethi, G. S., and Behl, R. K. 2004. Indices of drought tolerance in wheat genotypes at early stages of plant growth. *J. Agron. Crop Sci*. 190: 1-6.

Dhopte, A. M. and M. M. Livera. 1989. *Useful Techniques for Plant Scientists Published by Forum for Plant Scientists.* Forum for Plant Physiologist. Akola, Maharashtra, 329p.

Dhungana, S.K., Kim, H.S., Kang, B.K., Seo, J.H., Kim, H.T., Shin, S.O., Oh, J.H. and Baek, I.Y. 2021. Identification of QTL for Tolerance to Flooding Stress at Seedling Stage of Soybean (*Glycine max* L. Merr.). *Agronomy* [e-journal] 11(5). Available: https:// doi.org/10.3390/agronomy11050908. ISSN 2073-4395 [30 June 2021].

Ding, J., Liang, P., Guo, D., Liu, D., Yin, M., Zhu, M., Li, C., Zhu, X., and Guo, W.2020. Remedial application of urea eliminates yield losses in wheat waterlogged during stem elongation. *Agriculture* 10(1):23-36.

Dissanayake, I.A.J.K., Ranwala, S.M.W., and Perera, S.S.N.2019. Germination and seedling growth responses of Sri Lankan grown Sesame/Thila (*Sesamum indicum* L.) for simulated drought conditions. *J. Natl. Sci. Found. Sri Lanka* 47(4):479-490.

Dossa, K. 2016. A physical map of important QTLs, functional markers and genes available for sesame breeding programs. *Physiol. Mol. Biol. Plants* 22: 613–619.

Dossa, K., Diouf, D., Wang, L., Wei, X., Zhang, Y., Niang, M., Fonceka, D., Yu, J., Mmadi, M.A, Yehouessi, L., Liao, B., Zhang, X., Cisse, N., 2017. The emerging oilseed crop *Sesamum indicum* enters the "Omics" era. *Front. Plant Sci*. [e-journal] 8(1154). Available: https://doi.org/10.3389/fpls.2017.01154 ISSN 1664-462X [21 March 2019].

Dossa, K., Marie, A.M., Rong, Z., Qi, Z., Mei, Y., Ndiaga, C., Diaga, D., Linhai, W., and Xiurong, Z. 2018. The contrasting response to drought and waterlogging is underpinned by divergent DNA methylation programs associated with transcript accumulation in sesame. *Plant Sci.* 277:207-217.

Drew, M.C.1992. Soil aeration and plant root metabolism. *Soil Sci*. 154: 259-268.

ECOSTAT [Department of Economics and Statistics]. 2020. ECOSTAT home page [on line]. Available: http://www.ecostat.kerala.gov.in. [25 Dec. 2020].

El Harfi, M., Hanine, H., Rizki, H., Latrache, H., and Nabloussi, A.2016. Effect of drought and salt stresses on germination and early seedling growth of different color-seeds of sesame (*Sesamum indicum*). *Int. J. Agric. Biol*. 18(06):1088-1094.

Elekhtyar, N. M. 2015. Efficiency of *Pseudomonas fluorescens* as Plant Growth-Promoting Rhizobacteria (PGPR) for the enhancement of seedling vigor, nitrogen uptake, yield and its attributes of rice (*Oryza sativa* L.). *Int. J. Sci. Res. Agric. Sci*. 2:57-67.

El-Tayeb, M.A. 2005. Response of barley grains to the interactive effects of salinity and salicylic acid. *Plant Growth Reg*. 45: 215-224.

Evans, D. E. 2003. Aerenchyma formation. *New Phytol.* 161:35–49.

FAOSTAT [Food and Agriculture Organization Statistical Databases] 2020. FAOSTAT home page [on-line]. Available: http://faostat.fao.org. [08 Dec. 2019].

Farooq, M. and Hussain, M. 2017. Evaluating the role of seed priming in improving drought tolerance of pigmented and non-pigmented rice. *J. Agron. Crop Sci*. 203 (4): 269-276.

Farsiani, A. and Ghobadi, M. E. 2009. Effects of PEG and NaCl stress on two cultivars of corn (*Zea mays* L.) at germination and early seedling stages. *World Acad. Sci. Eng. Tech*. 57: 382- 385.

Folzer, H., Dat, J., Capelli, N., Rieffel, D., and Badot, P.M.2006. Response to flooding of sessile oak: An integrative study. *Tree Physiol*. 26: 759-766.

Fukao, T. and Bailey-Serres, J., 2004. Plant responses to hypoxia–is survival a balancing act? *Trends Plant Sci.* 9(9):449-456.

Garcia-Mata, C. and Lamattina, L. 2002. Nitric oxide and abscisic acid cross talk in guard cells. *Plant Physiol*. 128: 790–792.

Garrett, E.H. and Woodworth, R.S. 1969. *Statistics in Psychology and Education*. Vakils, Feffer and Simons Pvt. Ltd., Bombay, 329p.

Garthwaite, A.J., von Bothmer, R., and Colmer, T.D.2003. Diversity in root aeration traits associated with waterlogging tolerance in the genus Hordeum. *Funct. Plant Biol*. 30:875–889.

Gehlen, J., Panstruga, R., Smets, H., Merkelbach, S., Kleines, M., Porsch, P., Fladung, M., Becker, I., Rademacher, T., Hausler, R.E., and Hirsch, H.J., 1996. Effects of altered phosphoenolpyruvate carboxylase activities on transgenic C3 plant *Solanum tuberosum*. *Plant Mol. Biol.* 32(5):831-848.

Ghasemzadeh, A. and Jaafar, H.Z. 2013. Interactive effect of salicylic acid on some physiological features and antioxidant enzymes activity in ginger (*Zingiber officinale* Roscoe). *Molecules* 18(5):5965-5979.

Ghoi, K., Lee, L., Kim, S. D., and Hong, E. 1996. Dry matter production and seed yield of soyabean cultivars as affected by excessive water stress at the vegetative growth and flowering stages. *J. Agric. Sci*. 38(2): 117-122.

Gill, R. K., Sharma, A. D., Singh, P., and Bhullar, S. S. 2002. Osmotic stress induced changes in germination, growth and soluble sugar content of *Sorghum bicolor* (L.) Moench seeds. *Bulg. J. Plant. Physiol*. 28: 12- 25.

Glass, A.D.M. 1975. Inhibition of phosphate uptake in barley roots by hydroxy-benzoic acids. *Phytochem*. 14:2127- 2130.

GOK [Government of Kerala]. 2005. *Area production and productivity trends of important crops in Kerala (from 1985-86 to 2004-05)* [on-line].

Available:http://www.ecostat.kerala.gov.in/images/pdf/publications/Agriculture/data/old/area_crops_8586_0405.pdf [20 Aug 2020].

GOK [Government of Kerala]. 2014. *Agricultural Statistics 2012-2013* [on- line]. Available:http://www.ecostat.kerala.gov.in/images/pdf/publications/Agri culture /data/old/agristat_1213.pdf [25 Aug 2020].

GOK [Government of Kerala]. 2016. *An analytical study on agriculture in Kerala* [on-line].Available:http://www.ecostat.kerala.gov.in/images/pdf/publications/ReportsOtherDepts/Agriculture_OD/ASR_2817.pdf [05 June 2019].

GOK [Government of Kerala]. 2020a. *Agricultural Statistics 2018-19* [on-line]. Available:http://www.ecostat.kerala.gov.in/images/pdf/publications/Agriculture/data/2018-19/ agriculture_statistics_2018_19.pdf [28 April 2019].

GOK [Government of Kerala]. 2020b. *Land Utilization of Kerala in 2018-19* [on-line]. Available: http://www.ecostat.kerala.gov.in/index.php/agriculture [10 July 2020].

Golestani, M. and Pakniyat, H. 2007. Evaluation of drought tolerance indices in sesame lines. *J. Water Soil Sci.* 11(41):141–150.

Gonzalez, M. C., Sanchez, R., and Cejudo, F. J.2003. Abiotic stresses affecting water balance induce phosphoenolpyruvate carboxylase expression in roots of wheat seedlings. *Planta* 216**:**985–992.

Good, A.G. and Crosby, W.L. 1989. Anaerobic induction of alanine aminotransferase in barley root tissue. *Plant Physiol.* 90:1305-1309.

Goyal, K., Kaur, K., and Kaur, G., 2020. Foliar treatment of potassium nitrate modulates the fermentative and sucrose metabolizing pathways in contrasting maize genotypes under water logging stress. *Physiol. Mol. Biol. Plants* 26(5):899-906.

Grassini, P., Indaco, G.V., Pereira, M.L., Hall, A.J. and Trapani, N.2007. Responses to short-term waterlogging during grain filling in sunflower. *Field Crops Res*. 101(3):352-363.

Guo, W.Q., Chen, B.L., Liu, R.X., and Zhou, Z.G. 2010. Effects of nitrogen application rate on cotton leaf antioxidant enzyme activities and endogenous

hormone contents under short-term waterlogging at flowering and boll forming stage. *Chinese J. Appl. Ecol*. 21: 53-60.

Gutierrez Boem, F.H., Lavado, R.S., and Porcelli, C.A.1996. Note on the effects of winter and spring waterlogging on growth, chemical composition and yield of rapeseed. *Field Crop Res.* 47: 175-179.

Habibzadeh, F., Sorooshzadeh, A., Pirdashti, H., and Sanavy, S.A.M.M.2012. Effect of nitrogen compounds and tricyclazole on some biochemical and morphological characteristics of waterlogged-canola. *Int. Res. J. Appl. Basic Sci.* 3(1):77-84.

Habibzadeh, F., Sorooshzadeh, A., Pirdashti, H. and Sanavy, S. A. M. M. 2013.Alleviation of waterlogging damage by foliar application of nitrogen compounds and tricyclazole in canola. *Aust. J. Crop Sci.* 7(3):401-406.

Hageman, R.H. and Flesher, D. 1960. Nitrate reductase activity in corn seedlings as affected by light and nitrate content of nutrient media. *Plant Physiol*. 35(5): 700– 708.

Hasanuzzaman, M., Hossain, M.A., da Silva, J.A.T., and Fujita, M., 2012. Plant responses and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In: Bandi, V., Shanker, A.K., Shanker, C., and Mandapaka, M. (eds), *Crop Stress and its Management: Perspectives and Strategies*. Springer, Berlin, pp. 261–316.

Hassan, A. A. Karim, N. N, Sarkar, A.A., and Ali, M. H. 2001. Effect of waterlogging on the growth and yield of summer grown sesame *(Sesamum indicum)*. I*ndian J. Agric. Sci.* 71 (4): 271-2.

Hassanzadeh, M., Asghari, A., Jamaati-e-somarin, S., Saeidi, M., Zabihi-e-Mahmoodabad, R., and Hokmalipour, S. 2009a. Effects of water deficit on drought tolerance indices of Sesame (*Sesamum indicum* L.) genotypes in Moghan Region. *Res. J. Environ. Sci*. 3:116-121.

Hassanzadeh, M., Ebadi, M., Panahyan-e-Kivi, M., Jamaati-e-Somarin, S.H., Saeidi, M., and Zabihi-e-Mahmoodabad, R., 2009b. Evaluation of drought stress on relative water content and chlorophyll content of sesame (*Sesamum indicum L.*) genotypes at early flowering stage. *Res. J. Environ*. *Sci*. 3:345–350.

Heikal, M.M, Shaddad, M. A., and Ahmed, A. M. 1982. Effect of water stress and gibberellic acid on germination of flax, sesame and onion seed. *Biol. Plant.* 24:124-129.

Hellal, F.A., El-Shabrawi, H.M., Abd El-Hady, M., Khatab, I.A., El-Sayed, S.A.A. and Abdelly, C., 2018. Influence of PEG induced drought stress on molecular and biochemical constituents and seedling growth of Egyptian barley cultivars. *J. Genet. Eng. Biotechnol.* 16(1):203-212.

Helmeric, R. H. and Pfeifer, R. P. 1954. Differential varietal responses of winter wheat germination and early growth to controlled limited moisture conditions. *Agron. J*. 46:560-562.

Hemavathi, Upadhyaya, C.P., Akula, N., Kim, H. S., Jeon, J. H., Ho, O.M., Chun, S. C., Kim, D. H., and Park, S. W. 2010a. Biochemical analysis of enhanced tolerance in transgenic potato plants overexpressing D-galacturonic acid reductase gene in response to various abiotic stresses. *Mol. Breeding* 28(1):105-115.

Hemavathi, Upadhyaya, C.P., Akula, N., Young, K. E., Chun, S. C., Kim, D. H., and Park, S. W. 2010b. Enhanced ascorbic acid accumulation in transgenic potato confers tolerance to various abiotic stresses. *Biotechnol Lett* 32:321-330.

Hiscox, J. D. and Israelstam, G. F. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. C*an. J. Bot.* 57: 1332-1334.

Hoff, T., Stummann, B. M., and Henningsen, K. W. 1992. Structure function and regulation of nitrate reductase in higher plants. *Physiol. Plant.* 84 616–624.

Hossain, M. A and Salahuddhin, A. B. M.2001. Growth and yield of sesame in relation to population density. *Bangladesh J. Life Sci.* 6(1):59-65.

Hossain, M. M. 2006. Effect of waterlogging on morpho-physiological attributes and yield of sesame genotypes. M.Sc. (Bot.) thesis, Bangladesh Agricultural University, Mymensingh, 45p.

Hsu, F.H., Lin, J.B., and Vhang, S.R.2000. Effects of waterlogging on seed germination. *Bot. Bull. Acad. Sin*. 41:267–273.

Huang, B., Su, J., Zhang, G., Luo, X., Wang, H., Gao, Y., Ma, G., Wang, J., Cai, D., Zhang, X. and Huang, B., 2017. Screening for Eruca genotypes tolerant to

polyethylene glycol-simulated drought stress based on the principal component and cluster analyses of seed germination and early seedling growth. *Plant Genetic Resour.* 15(2):187-193.

Hurng, W.P. and Kao, C.H. 1994. Lipid peroxidation and antioxidative enzymes in senesencing tobacco leaves following flooding. *Plant Sci*. 96:41–4.

Hussain, A., Zafar, U. Z., and Habibur, R. A. 2014. Flooding tolerance in cotton (*Gossypium hirsutum* L.) at early vegetative and reproductive growth stages. *Pakist*. *J. Bot.* 46(3): 1001-1009.

Hwang, S.Y., Lin, H.W., Chern, R.H., Lo, H.F., and Li, L. 1999. Reduced susceptibility to waterlogging together with high-light stress is related to increases in superoxide dismutase and catalase activities in sweet potato. *Plant Growth Regul*. 27:167–72.

Idrissi, O., Udupa, S.M., De Keyser, E., McGee, R.J., Coyne, C.J., Saha, G.C., Muehlbauer, F.J., Van Damme, P., and De Riek, J. 2016. Identification of quantitative trait loci controlling root and shoot traits associated with drought tolerance in a lentil (*Lens culinaris* Medik.) recombinant inbred line population. *Front. Plant Sci*. [e-journal] 7(3). Available: https://www.frontiersin.org/articles/10.3389/fpls.2016.01174/full. ISSN [10 June 2019].

IOPEPC [Indian Oilseeds and Produce Export Promotion Council].2017. IOPEPC home page [on line]. Available: http://www.iopepc.org. [15 Dec.2018].

ISTA.1999. International rules for seed testing. *Seed Sci. Technol*. (Supplement Rules).27: 25-30.

Izui, K., Matsumura, H., Furumoto, T., and Kai, Y.2004. Phosphoenolpyruvate carboxylase: a new era of structural biology. *Annu. Rev. Plant Biol.* 55**:**69–84.

Jain, R., Singh, S.P., Singh, A., Singh, S., Chandra, A., and Solomon, S. 2015. Response of foliar application of nitrogen compounds on sugarcane grown under waterlogging stress. *Sugar Tech*. 18(4):433-436.

Jiang, Z., Song, X. F., Zhou, Z. Q., Wang, L. K., Li, J. W., and Deng, X. Y. 2010. Aerenchyma formation: programmed cell death in adventitious roots

of winter wheat (*Triticum aestivum*) under waterlogging. *Funct. Plant Biol.* 37:748–755.

Juenger, T.E. 2013. Natural variation and genetic constraints on drought tolerance. *Curr. Opin. Plant Biol*. 16: 274–281.

Kadkhodaie, A., Zahedi, M., Razmjoo, J., and Pessarakli, M. 2014. Changes in some anti-oxidative enzymes and physiological indices among Sesame genotypes (*Sesamum indicum* L.) in response to soil water deficits under field conditions. *Acta Physiol. Plant*. 36:641-650.

Kalefetoglu, M. T., Turan, O., and Ekmekci, Y.2009. Effect of water deficit induced by PEG and NaCl on chickpea (*Cicer arientinum* L.) cultivar and lines at early seedling stage. *G.U. J. Sci.* 22(1):5-14

Kamoutsis, A.P. and Chronopoulou-Sereli, A.G. 1999. Paclobutrazol affects growth and flower bud production in gardenia under different light regimes. *Hortic. Sci.* 34: 674– 675.

Kassab, O.M., Mehanna, H.M., and Aboelill, A. 2012. Drought impact on growth and yield of some sesame varieties. *J. Appl. Sci. Res.* 8:4544–4551.

Kato, C., Ohshima, N., Kamada, H., and Satoh, S.2001.Enhancement of the inhibitory activity for greening in xylem sap of squash root with waterlogging. *Plant Physiol. Biochem.* 39:513–519.

Khan, M.N., Zhang, J., Luo, T., Liu, J., Ni, F., Rizwan, M., Fahad, S., and Hu, L., 2019. Morpho-physiological and biochemical responses of tolerant and sensitive rapeseed cultivars to drought stress during early seedling growth stage. *Acta physiol. Plant.* [e-journal] 41(2). Available: https://doi.org/10.1007/s11738-019-2812-2. ISSN 1861-1664 [20 March 2020].

Khayatnezhad, M., Gholamin, R., Jamaatie-Somarin, S.H., and Zabihi-Mahmoodabad, R. 2010. Effects of PEG stress on corn cultivars (*Zea mays* L.) at germination stage. *World Appl. Sci. J*. 11(5): 504-506.

Khodarahmpour, Z. 2011. Effect of drought stress induced by polyethylene glycol on germination indices in corn (*Zea mays* L.) hybrids. *Afr. J. Biotechnol*. 10(79): 18222-18227.

Kilen, T. C. and Andrew, R. H. 1969. Measurement of drought resistance in corn. *Agron. J.* 61:669-672.

Kishorekumar, A., Abdul Jaleel, C., Manivannan, P., Sankar, B., Sridharan, R., Murali, P.V., and Panneerselvam, R.2008. Comparative effects of different triazole compounds on antioxidant metabolism of *Solenostemon rotundifolius*. *Colloids Surf. B*. 62: 307-311.

Kizil, S. and Engin, Y.O.L.2018. Influence of salinity and drought stresses on seed germination and seedling growth characteristics in sesame (*Sesamum indicum* L.). *Mediterr. Agric. Sci.* 31(2):155-160.

Klinghammer, M. and Tenhaken, R. 2007. Genome-wide analysis of the UDP-glucose dehydrogenase gene family in Arabidopsis, a key enzyme for matrix polysaccharides in cell walls. *J. Exp. Bot.* 58:3609–3621.

Kmal, A.K., Amen, E.A., and Al-Said, A.M. 2006. Response of snap bean (*Phaseolus vulgaris* L.) to some salicylic acid derivatives and selenium under high temperature stress. *J. Agric. Sci. Mansoura Univ*. 31(11): 7321-7328.

Koca, H., Bor, M., Ozdemir, F., and Turkan, G. 2007. The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environ. Exp. Bot*. 60:344-351.

Kosturkova, G., Todorova, R., Sakthivelu, G., Akitha Devi, M.K., Giridhar, P., Rajasekaran, T., and Ravishankar, G.A., 2008. Response of Bulgarian and Indian soybean genotypes to drought and water deficiency in field and laboratory conditions. *Gen. Appl. Plant Physiol*. 34(3):239-250.

Kuluev, B. R. Musin, K. G., Berezhneva, Z. A., Mikhaylova, E. V., and Zaikina, E. A. 2019. Role of the expansin and xyloglucan endotransglycosylase genes in the regulation of tobacco growth under the influence of abiotic stress factors. In: Kochetov, A. and Salina, E. (eds), *Proceedings of the Fifth International Scientific Conference PlantGen2019*, 24–29 June 2019, Novosibirsk. Russian Academy of Sciences, Novosibirsk State University, pp. 135–136.

Kumaraswamy, H.H., Jawaharlal, J., Ranganatha, A.R.G. and Rao, S.C. 2015. Safe sesame (*Sesamum indicum* L.) production: Perspectives, practices and challenges. *J. Oilseeds Res*. 32(1): 1-24.

Lambers, H., Chapin, I. F. S., and Pons, T. L. 2008. *Plant Physiological Ecology* [e-book]. Dept. of Natural and Agricultural Sciences at the University of Western Australia. Available:http://www.esalq.usp.br/lepse/imgs/conteudo_thumb/Plant-Physiological-Ecology-by-Hans-Lambers--2008-.pdf. [30 Oct. 2019].

Leary, B.O., Park, J., and Plaxton, W. C. 2011.The remarkable diversity of plant PEPC (Phosphoenolpyruvate carboxylase): recent insights into the physiological functions and post-translational controls of non-photosynthetic PEPCs. *Biochem. J.* 436**:**15–34.

Leul, M. and Zhou, W. 1998. Alleviation of waterlogging damage in winter rape by application of uniconazole: Effects on morphological characteristics, hormones and photosynthesis. *Field Crop Res*. 59: 121–127.

Li, C., Jiang, D., Wollenweber, B., Li, Y., Dai, T., and Cao, W., 2011. Waterlogging pretreatment during vegetative growth improves tolerance to waterlogging after anthesis in wheat. *Plant Sci.* 180(5):672-678.

Li, D., Liu, P., Yu, J., Wang, L., Dossa, K., Zhang, Y., Zhou, R., Wei, X., and Zhang, X., 2017. Genome-wide analysis of WRKY gene family in the sesame genome and identification of the WRKY genes involved in responses to abiotic stresses. *BMC Plant Biol.* 17(1):1-19.

Lin, K.H.R., Tsou, C.C., Hwang, S.Y., Chen, L.F.O., and Lo, H.F. 2006. Paclobutrazol pre-treatment enhanced flooding tolerance of sweet potato. *J. Plant Physiol*. 163, 750–760.

Lisko, K.A., Torres, R., Harris, R.S., Belisle, M., Vaughan, M.M., and Jullian, B. 2013.Elevating vitamin C content via overexpression of myo-inositol oxygenase and l-gulono-1,4-lactone oxidase in Arabidopsis leads to enhanced biomass and tolerance to abiotic stresses. *In Vitro Cell Dev. Biol.* Plant.49:643-55.

Lopez-Cantarero, I., Ruiz, J.M., Hernandez, J., and Romero, L. 1997. Nitrogen metabolism and yield response to increase in nitrogen-phosphorus fertilization: Improvement in green house cultivation of eggplant (*Solanum melongena*). *J. Agric. Food Chem*. 45:4227-4231.

Lorence, A., Chevone, B.I., Mendes, P., and Nessler, C.L. 2004.Myo-inositol oxygenase offers a possible entry point into plant ascorbate biosynthesis. *Plant Physiol.*134:1200-5.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. 1951. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* 193: 265-275.

Luan, H., Guo, B., Pan, Y., Lv, C., Shen, H., and Xu, R. 2018.Morpho-anatomical and physiological responses to waterlogging stress in different barley (*Hordeum vulgare* L.) genotypes. *Plant Growth Regul*. 85:399–409.

Lyon, C.K. 1972. Sesame: Current knowledge of composition and use. *J. Am. Oil Chem. Soc*. 49(4):245–249.

Maehly, A.C. 1954. The assay of catalase and peroxidases. In: Glick, D. (ed.), *Methods of Biochemical Analysis*. Inter-science publishers, New York, pp. 357-424.

Maguire, J. D. 1962. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. *Crop Sci*. 2(2): 176-177.

Malik, A.I., Colmer, T.D., Lamber, H., and Schortemeyer, M.2001. Changes in physiological and morphological traits of roots and shoots of wheat in response to different depths of waterlogging. *Aust. J. Plant. Physiol*.28: 1121-1131.

Mano, Y. and Omori, F. 2007. Breeding for flooding tolerant maize using “teosinte” as a germplasm resource. *Plant Root* 1: 17-21.

Marschner, H.1995. Mineral nutrition of higher plants. *Ann. Bot*. 78(4):527-528.

Marschner, P. and Timonen, S.2006. Bacterial community composition and activity in rhizosphere of roots colonized by arbuscular mycorrhizal fungi. In: Mukerji, K.S. C. Manoharachary, C., and Singh, J. (eds), *Microbial activity in the Rhizosphere*. Springer, Berlin, pp 139–154.

Martin, N.H., Bouck A. C., and Arnold, M. L. 2006. Detecting adaptive trait introgression between *Iris fulva* and *Iris brevicaulis* in highly selective field conditions. *Genet*. 17(2): 2481-2489.

Mattana, M., Bertani, A., and Reggiani, R. 1994. Expression of glutamine synthetase during the anaerobic germination of *Oryza sativa* L. *Planta* 195:147–149.

Mensah, J. K., Obadoni, B. O., Eruotor, P. G., and Onome-irieguna, F. 2009. Simulated flooding and drought effects on germination, growth, and yield parameters of Sesame (*Sesamum indicum* L.). *Afr. J. Biotechnol.* 5: 1249-1253.

Mensah, J. K., Obadoni1, B. O., Eruotor, P.G., and Onome-Irieguna, F. 2006. Simulated flooding and drought effects on germination, growth, and yield parameters of sesame (*Sesamum indicum* L.). *Afr. J. Biotechnol.* 5 (13):1249-1253.

Mmadi, M.A., Dossa, K., Wang, L., Zhou, R., Wang, Y., Cisse, N., Sy, M.O., and Zhang, X., 2017. Functional characterization of the versatile MYB gene family uncovered their important roles in plant development and responses to drought and waterlogging in sesame. *Genes* [e-journal] 8(12). Available: https://doi.org/10.3390/genes8120362. ISSN 2073-4425 [24 May 2019].

Mohammadkhani, N. and Heidari, R 2008. Water stress induced by polyethylene glycol 6000 and sodium chloride in two corn cultivars. *Pakistan J. Biol. Sci.* 11(1): 92-97.

Morello, L., Giani, S., and Breviaro, D. 1994. The influence of anaerobiosis on membrane-associated rice (*O. sativa* L.) protein kinase activities. *J. Plant Physiol.* 144:500-504.

MSSRF [M.S. Swaminathan Research foundation].2007. MSSRF home page [on-line]. Available: http:// www.mssrf.org. [07 Dec. 2018].

Munir, S., Mumtaz, M. A., Ahiakpa, J. K., Liu, G., Chen, W., Zhou, G. Zheng, W. Ye, Z. and Zhang, Y. 2020. Genome-wide analysis of Myo-inositol oxygenase gene family in tomato reveals their involvement in ascorbic acid accumulation. *BMC Genomics*. 21:284-299.

Myint, D., Gilani, S. A., Kawase, M., and Watanabe, N. K. 2020. Sustainable Sesame (Sesamum indicum L.) Production through Improved Technology: An Overview of Production, Challenges, and Opportunities in Myanmar. *Sustainability* [e-journal] 12 (9). Available: https://doi.org/10.3390/su12093515. ISSN 2071-1050 [25 March 2020].

Najeeb, U., Mirza, M.Y., Jilani, G., Mubashir, A.K. and Zhou, W.J.2012. Sesame. In: Gupta, S.K. (ed.), *Technological Innovations in Major World Oil Crops*. Springer-Verlag New York, USA, pp. 131–145.

Nakagawa, T., Izumi, T., Banba, M., Umehara, Y., Kouchi, H., Izui, K., and Hata, S.2003.Characterization and expression analysis of genes encoding phosphoenolpyruvate carboxylase and phosphoenolpyruvate carboxylase kinase of *Lotus japonicas*, a model legume. *Mol. Plant Microbe Interact.* 16:281-288.

Nandi, S., Subudhi, P.K., Senadhira, D., Manigbas, N.L., SenMandi, S., and Huang, N. 1997. Mapping QTLs for submergence tolerance in rice by AFLP analysis and selective genotyping. *Mol. Gen. Genet*. 255(1): 1-8.

Nguyen, H. C., Lin, K. H., Ho, S. L., Chiang, C. M., Yang, C. M. 2018a. Enhancing the abiotic stress tolerance of plants: From chemical treatment to biotechnological approaches. *Physiol. plant.* 164:452–466.

Nguyen, T. N., Tuan, P. A., Mukherjee, S., Son S., and Ayele, B. T. 2018b. Hormonal regulation in adventitious roots and during their emergence under waterlogged conditions in wheat. *J. Exp. Bot.* 69 4065–4082.

Nicholas, J. C., Harper, J., and Hageman, R. H. 1976. Nitrate reductase activity in Soybeans (*Glycine max* L.). *Plant Physiol.* 58: 736.

Noreen, S., Fatima, Z., Ahmad, S., and Ashraf, M. 2018. Foliar application of micronutrients in mitigating abiotic stress in crop plants plant nutrients and abiotic stress tolerance. In: Hasanuzzaman, M. (ed.), *Plant Nutrients and Abiotic Stress Tolerance*, Springer, Singapore, pp.95–117.

Norman, H., Cocks, P., and Galwey, N.2002. Hardseededness in annual clovers: Variation between populations from wet and dry environments. *Crop Pasture Sci*. 53: 821–829.

Okcu, G., Kaya, M.D., and Atak, M. 2005. Effects of salt and drought stresses on germination and seedling growth of pea (*Pisum Sativum* L.). *Turkish J. Agric. For.* 29:237-242.

Olgun, M., Kumlay, A.M., Adiguzel, M.C., and Caglar, A.2008. Effects of waterlogging in wheat (*T. aestivum* L.). *Acta Agric. Scand. B Soil Plant Sci*. 58: 193-198.

Pang, J., Newman, I., Mendham, N., Zhou, M., and Shabala, S. 2006. Microelectrode ion and O_2 fluxes measurements reveal differential sensitivity of barley root tissues to hypoxia. *Plant Cell Environ*. 29:1107–1121.

Pang, J., Ross, J., Zhou, M., Mendham, N., and Shabala, S. 2007. Amelioration of detrimental effects of waterlogging by foliar nutrient sprays in barley. *Funct. Plant. Biol*. 34(3):221-227.

Parelle, J., Dreyer, E., and Brendel, O. 2010. Waterlogging signalling and tolerance in plants. *Agric. Sci. Digest* 9(4):241-265.

Parelle, J., Dreyer, E., and Brendel, O.2010. Genetic variability and determinism of adaptation of plants to soil waterlogging. In: Mancuso, S. and Shabala, S (eds), *Waterlogging Signalling and Tolerance in Plants*. Springer Berlin Heidelberg, pp. 241-265.

Partheeban, C., Chandrasekhar, C.N., Jeyakumar, P., Ravikesavan, R., and Gnanam, R. 2017. Effect of PEG induced drought stress on seed germination and seedling characters of maize (*Zea mays* L.) genotypes. *Int. J. Curr. Microbiol. Appl. Sci.* 6(5):1095-1104.

Pavli, O.I., Foti, C., Skoufogianni, G., Karastergiou, G., Panagou, A., and Khah, E.M. 2020. PEG-Induced Drought Stress During Germination Effects on Soybean Germplasm. *Agric. Res. Technol.* 23(5):70-80.

Perata, P. and Alpi, A. 1993. Plant responses to anaerobiosis. *Plant Sci*. 93:1–17.

Qiu, F., Zheng, Y., Zhang, Z., and Xu S. Mapping of QTL Associated with Waterlogging Tolerance during the Seedling Stage in Maize. *Ann. Bot*. 99(6): 1067–1081.

Radhamani, T., Ushakumari, R., Amudha, R., and Anjani, K. 2012. Response to water stress in castor (*Ricinus communis* L.) genotypes under in vitro conditions. *J. Cereals Oilseeds* 3(4):56-58.

Rai, R.K., Srivastava, J.P., and Shahi, J.P. 2004. Effects of waterlogging on some biochemical parameters during early growth stages of maize. *Indian J. Plant Physiol*. 9: 65-68.

Rajendran, R. A., Muthiah, A. R., Manickam, A., Shanmugasundaram, P., and John Joel, A.2011. Indices of drought tolerance in sorghum (*Sorghum bicolor* L. Moench) genotypes at early stages of plant growth. *Res. J. Agric. Biol. Sci*. 7: 42-46.

Rajhi, I. and Mhadhbi, H., 2019. Mechanisms of aerenchyma formation in maize roots. *Afr. J. Agric. Res.* 14(14):680-685.

Ramu, S.V., Rohini, S., Keshavareddy, G., Gowri, N.M., Shanmugham, N. B., Kumar, A.R.V., Sarangi, S.K., Anand, K.P., and Udayakumar, M.2012. Expression of a synthetic cry1AcF gene in transgenic Pigeon pea confers resistance to *Helicoverpa armigera*. *J. Appl. Entomol*. 136 (9): 675-687.

Ranjbarfordoei, A. R., Samson, P., Damme, V., and Lameur, R. 2000. Effect of drought stress induced by polyethylene glycol on pigment content and photosynthetic gas exchange of *Pistacia khinjuk* and *P. mutica. Photosynthetica* 38:443-447.

Rao, R., Li, Y., and Bryan, H.H.2002. Assessment of foliar sprays to alleviate flooding injury in corn (*Zea mays* L.). *Proc. Florida State Hort. Soc*. 115: 208-211.

Raskin, I., Skubatz, H., Tang, W., Meeuse, B.J.D. 1990. Salicylic acid levels in thermogenic and non-thermogenic plants. *Ann. Bot*.66:376-373.

Rauf, S. 2008. Breeding sunflower (*Helianthus annuus* L.) for drought tolerance. *Commun. Biometry Crop Sci*. 3(1): 29-44.

Rauf, S., Sadaqat, H. A., and I. A. Khan, I. A.2008. Effect of moisture regimes on combining ability variations of seedling traits in sunflower (*Helianthus annuus* L.). *Candian J. Plant Sci*. 88: 323-329.

Ren, B., Dong, S., Zhao, B., Liu, P., and Zhang, J. 2017. Responses of nitrogen metabolism, uptake and translocation of maize to waterlogging at different growth stages. *Front. Plant sci.* [e-journal]. 8 (1). Available: https://doi.org/10.3389/fpls.2017.01216. ISSN 1664-462X [21 March 2019].

Ren, B., Zhang, J., Dong, S., Liu, P. and Zhao, B. 2018. Exogenous 6-benzyladenine improves antioxidative system and carbon metabolism of summer maize waterlogged in the field. *J. Agron. Crop Sci.* 204: 175–184.

Ren, B., Zhu, Y., Zhang, J., Dong, S., Liu, P., and Zhao B. 2016. Effects of spraying exogenous hormone 6-benzyladenine (6-BA) after waterlogging on grain yield and growth of summer maize. *Field Crops Res.* 188:96–104.

Rogers, S.O. and Bendich, A.J., 1985. Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. *Plant Mol. Biol*. 5(2):69-76.

Saab, I.N. and Sachs, M.M.1996.A flooding-induced xyloglucan endo-transglycosylase homolog in maize is responsive to ethylene and associated with aerenchyma. *Plant Physiol*.112:385–391.

Sachs, M.M., Freelins, M., and Okimoto, R. 1980. The anaerobic proteins of maize. *Cell* 20: 761–767.

Saddam, S., Bibi, A., Sadaqat, H. A., and Usman, B. F. 2014. Comparison of 10 sorghum (*Sorghum bicolor* L.) genotypes under various water stress regimes. *J. Animal Plant Sci.* 24(6):1811-1820.

Saha, R. R, Ahmed, F., Mokarroma, N., Rohman, M.M., and Golder, P.C. 2016. Physiological and biochemical changes in Waterlog tolerant sesame genotypes. *SAARC J. Agri.* 14(2): 31-45.

Saleem, M., Arshad, M., Hussain, S., and Bhatti, A.S.2007. Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. *J. Ind. Microbiol. Biotechnol*. 34: 635–648.

Sanaa, Z.A.M., Ibrahim, S.I. and Sharaf-Eldeen, H.A.M. 2001. The effect of naphthaline acetic acid (NAA), salicylic acid (SA) and their combination on growth, fruit setting yield and some correlated components in dry bean (*Phaseolus vulgaris* L.). *Ann. Agric. Sci.* 46(2): 451-463.

Sanchez, R., Flores, A., and Cejudo, F.J.2006. Arabidopsis phosphoenolpyruvate carboxylase genes encode immunologically unrelated polypeptides and are differentially expressed in response to drought and salt stress. *Planta* 223:901–909.

Sangwan, R.S., Singh, N., and Plaxton, W.C.1992.Phosphoenolpyruvate carboxylase activity and concentration in the endosperm of developing and germinating castor oil seeds. *Plant Physiol*. 99:445–449.

Saravanakumar, D., Kavino, M., Raguchander, T., Subbian, P. and Samiyappan, R. 2011. Plant growth promoting bacteria enhance water stress resistance in green gram plants. *Acta Physiol. Plant.* 33:203–209.

Sarkar, P. K., Khatun, A., and Singha, A. 2016. Effect of Duration of Water-logging on Crop Stand and Yield of Sesame. *Int. J. Innov. Appl. Stud*. 14:1-6.

Satomi, S., Takafumi, N., Rika, M., Sayuri, K., Masao, W., Masao, I., Satoshi, S., Kyuya, H., Ryoj, T., and Toshihiro, M. 2014. Variation in root development response to flooding among 92 soybean lines during early growth stages. *Plant Prod. Sci.* 17(3):228-236.

Sebei, K., Ouerghi, Z., Kallel, H., and Boukhchina, S.2006.Evolution of phosphoenolpyruvate carboxylase activity and lipid content during seed maturation of two spring rapeseed cultivars (*Brassica napus* L.). *C. R. Biol*. 329:719–725.

Sepehr, M.F., Ghorbanli, M., and Amini, F., 2012. The effect of water stress on nitrate reductase activity, and nitrogen and phosphorus contents in *Cuminum cyminum* L. *Pak. J. Bot*. 44(3):899-903.

Setter, T.L., Waters, I., Sharma, S.K., Singh, K.N., Kulshreshtha, N., Yaduvanshi, N.P.S., Ram, P.C., Singh, B.N., Rane, J., and McDonald, G.2009. Review of wheat improvement for waterlogging tolerance in Australia and India: the importance of anaerobiosis and element toxicities associated with different soils. *Ann. Bot*. 103:221–235.

Shakirova F. M., Sakhabutdinova A. R., Bezrukova M. V., Fatkhutdinova R. A., Fatkhutdinova D. R. (2003). Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Sci.* 164 317–322.

Shakirova, F.M., Sakhabutdinova, A.R., Bezrukova, V., Fatkhutdinova, R.A., Fatkhutdinova, D.R. 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Sci.* 164:317-322.

Sharma, N. K. 2015. Molecular characterization and assessment of physiological and biochemical impact of biological and chemical elicitors on *Withania somnifera.* Ph. D. (Biotech.) thesis, Kumaun University, Nainital, Uttarakhand. 246p.

Sharma, V. P. 2014. *Oilseed production in India- Problems and prospects* [e- book]. Springer. Available: https://www.springer.com/gp/book/9788132237167. [30 Oct. 2019].

Singh, S. K., Singh, A. K., and Dwivedi, P. 2017. Modulating effect of salicylic acid on tomato plants in response to waterlogging stress. *Int. J. Agric. Environ. Biotechnol*. 10(1):31-37.

Sinha, B.K., Hague, H., and Pandey, O.P. 1995. Metabolic modification in *Zea mays* during waterlogging. *Plant Physiol. Biochem.* 22(2): 173–177.

Smethurst, C.F., Garnett, T., Shabala, S., Chrisitiane, F.S. and Sergey, S.2005 Nutritional and chlorophyll fluorescence responses of lucerne (*Medicago sativa*) to waterlogging and subsequent recovery. *Plant and Soil* 27:31-45.

Smirnoff, N., Conklin, P.L., and Loewus, F.A. 2001.Biosynthesis of ascorbic acid in plants: a renaissance. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. 52:437–67.

Smok, M.A., Chojnowski, M., Corbineau, F., and Come, D.1993. Effects of osmotic treatments on sunflower seed germination in relation with temperature and oxygen. In: Come, D. and Corbineau, F. (eds*), Proceeding of the 4th International Workshop on seed: Basic and Applied Aspects of Seed Biology*. Angers, France, pp. 1033-1038.

Snowden, R.E.D. and Wheeler, B.D.1993. Iron toxicity to fern plant species. *J. Ecol*. 81:35–46.

Springer, B., Werr, W., Starlinger, P., Bennett, D. C., Zocolika, M., and Freeling, M.1986. The Shrunken gene on chromosome 9 of *Zea mays* L. is expressed in various plant tissues and encodes an anaerobic protein. 205(3):461-8.

Sreepriya, S. and Girija, T. 2018. Seed priming for improving the weed competitiveness in sesame. *J. Crop Weed* 14(2): 40-45.

Statista.2020.Statista home page [on line]. Available: https://www.statista.com/statistics /769700/ india-sesame-production-volume. [07 Dec 2020].

Steffens, B., Geske, T., and Sauter, M.2010. Aerenchyma formation in the rice stem and its promotion by H_2O_2. *New Phytol.* 190:369-378.

Sudama, S., Tiwari, T. N., Srivastava, R. P., Singh G. P., and Singh S. 1998. Effect of potassium on stomatal behaviour, yield and juice quality of sugarcane under moisture stress condition. *Indian J. Plant Physiol.* 3:303–305.

Sun, H., Zhou, S., Song, X., Fu, Z., Chen, G., and Zhang, J. 2016. CH_4 emission in response to water-saving and drought-resistance rice (WDR) and common rice varieties under different irrigation managements. *Water Air Soil Pollut*. 227:1-12.

Sung, F. J. M. and Sun, Y. W.1990. Seasonal patterns of nitrate reductase and nitrogenase activities in *Arachis hypogaea*. *Field Crops Res.* 25:215–222.

Taiz, L. and Zeiger, E. 2010. *Plant Physiology* (5th Ed.). Sinauer Associates Inc. Publishers, Sunderland, 782p.

Tanesaka, E., Umeda, E., Kajiwara, A., Yamamoto, M., Masuda, K., Yamada K. 2011. Seed germination of sesame (*Sesamum indicum* L.) and dormancy break of its wild relative *S. mulayanum* Nair. *J. Crop Res*. 56:79–82.

Tanesaka, E., Umeda, E., Yamamoto, M., Masuda, K., Yamada, K., and Yoshida, M., 2012. Inheritance mode of seed dormancy in the hybrid progeny of sesame, *Sesamum indicum*, and its wild relative, *Sesamum mulayanum* Nair. Weed boil. Manag. 12(2):91-97.

Tong, C., Hill, C.B., Zhou, G., Zhang, X.Q., Jia, Y., and Li, C. 2021.Opportunities for improving waterlogging tolerance in cereal crops-physiological traits and genetic mechanisms. *Plants* [e-journal] 10(1560).Available: https://doi.org/10.3390/ plants10081560. ISSN 2223-7747 [30 July 2021].

Toojinda, T., Siangliw, M., Tragoonrung, S., and Vanavichit, A. 2003.Molecular genetics of submergence tolerance in rice: QTL analysis of key traits. *Ann. Bot.* 91:243–253.

Tripathy, S.K., Kar, J., Sahu, D. 2019. Advances in Sesame (*Sesamum indicum* L.) breeding. In: Al-Khayri, J.M., Jain, S.M., and Johnson, D.V. (eds), *Advances in Plant Breeding Strategies: Industrial and Food Crops.* Springer, Cham, Switzerland, pp. 577–635.

Ucan, K. and Killi, F. 2010. Effects of different irrigation programs on flower and capsule numbers and shedding percentage of sesame. *Agric. Water Manag*. 98: 227-233.

Ucan, K., Kıllı, F., Genco, G.C., and Merdun, H.2007. Effect of irrigation frequency and amount on water use efficiency and yield of sesame (*Sesamum indicum* L.) under field conditions. *Field Crop. Res*. 101:249–258.

Ushimaro, T., Shibasaka, M., and Tsuji, H. 1992. Development of O_2-detoxification system during adaptation to air of submerged rice seedlings. *Plant Cell Physiol*. 33: 1065-1071.

Van den Berg, L. and Zeng, Y. J.2006. Response of South African indigenous grass species to drought stress induced by polyethylene glycol (PEG) 6000. *S. Afr. J. Bot*. 72:284-286.

Varadachari, C. and Goertz, H.M. 2010. *Slow-release and Controlled-release Nitrogen Fertilizers.* ING Bulletins on Regional Assessment of Reactive Nitrogen Series No. 11, Angkor Publishers, New Delhi, 49p.

Vassault, A. 1983. Lactate dehydrogenase: UV-method with pyruvate and NADH. In: Bergmeyer, H.U. (ed.), *Methods of Enzymatic Analysis* (3rd Ed.). Verlag Chemie, Weinheim, Florida, pp.118-126.

Verslues, P. E., Agarwal, M., Katiyar-Agarwal, S., Zhu, J., and Zhu, J. K. 2006. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *Plant J.* 45:523-539.

Vignesh, M., Prakash, M., Priyadharshini, B., and Anandan, R. 2018. Multivariate analysis of sesame genotypes under induced drought conditions. *Int. J. Curr. Microbiol. App. Sci.* 7(7): 4062-4070.

Visser, E.J.W. and Voesenek, L.A.C.J.2005. Acclimation to soil flooding sensing and signal-transduction. *Plant Soil* 274:197–214.

Voesenek, L.A.C.J., Benschop, J.J., Bou, J., Cox, M.C.H., Vreeburg, R.A.M., and Peeters, A.J.M.2003. Interactions between plant hormones regulate submergence-induced shoot elongation in the flooding-tolerant dicot *Rumex palustris*. *Ann. Bot.* 91:205–211.

Wang, L., Li, D., Zhang, Y., Gao, Y., Yu, J., Wei, X., and Zhang, X. 2016. Tolerant and susceptible sesame genotypes reveal waterlogging stress response patterns. *PLOS ONE* [e-journal]11(3). Availbale: https://doi.org/10.1371/journal.pone. 0149 912. ISSN 1932-6203 [14 May 2019].

Wang, L., Lu, Z. P., Zhang, Y. X., Li, D. H., Lu, H. X., Zhang, X. Y., and Zhang, X. R. 2010. Isolating waterlogging tolerance related genes from sesame (*Sesamum indicum* L.) by suppression subtractive hybridization. *Chinese J. Oil Crop Sci.* 32 (4): 485- 490.

Wang, L., Yu, S., Tong, C., Zhao, Y., Liu, Y., Song, C., Zhang, Y., Zhang, X., Wang, Y., Hua, W. and Li, D., 2014. Genome sequencing of the high oil crop sesame provides insight into oil biosynthesis. *Genome biol.* 15(2):1-13.

Wang, L., Zhang, Y., and Qi, X. 2012. Global gene expression responses to waterlogging in roots of sesame (*Sesamum indicum* L.). Acta Physiol. Plant. 34:2241–2249.

Wang, L.J., Fan, L., Loescher, W., Duan, W., Liu, G.J., Cheng, J.S., Luo, H.B., and Li, S.H., 2010. Salicylic acid alleviates decreases in photosynthesis under heat stress and accelerates recovery in grapevine leaves. *BMC plant Biol.* 10(1):1-10.

Waters, I., Morell, S., Greenway, H., and Colmer, T.D., 1991. Effect of anoxia on wheat seedlings. II. Influence of O_2 supply prior to anoxia on tolerance to anoxia, alcoholic fermentation, and sugar levels. *J. Exp. Bot.* 42:1437–1447.

Watson, G.W. and Himelick, E.B.2004. Effects of soil pH, root density and tree growth regulator treatments on pin oak chlorosis. *J. Arboriculture*. 30:172–7.

Wei, W., Li, D., Wang, L., Ding, X., Zhang, Y., Yuan, G., and Zhang, X. 2013. Morpho-anatomical and physiological responses to waterlogging of sesame (*Sesamum indicum* L.). *Plant Sci.* 208: 102-111.

Wei, X., Liu, K., Zhang, Y., Feng, Q., Wang, L., Zhao, Y., Li, D.2015.Genetic discovery for oil production and quality in sesame. *Nat. Commun*. 6:1-10.

Williams, T. V., Snell, R. S., and Ellis, J. F.1967. Methods of measuring drought tolerance in corn. *Crop Sci*. 7:179-182.

Wu, F. B., Zhang, G. P., and Dominy, P. 2003. Four barley genotypes respond differently to cadmium: lipid peroxidation and activities of antioxidant capacity. *Environ. Exp. Bot.* 50: 67–78.

Wu, H., Xiang, J., Chen, H., Zhang, Y., Zhang, Y., and Zhu, F. 2018. Effects of exogenous growth regulators on plant elongation and carbohydrate consumption of rice seedlings under submergence. *J. Appl. Ecol.* 29, 149–157.

Wu, S.C., Cao, Z.H., Li, Z.G., Cheung, K.C., and Wong, M.H.2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. *Geoderma* 125: 155–166.

Xing, X. H., fang, C. W., long, I. I., jiang, H. Q., Qin, Z. H. O. U., Jiang, H. D., and Wang, S. H. 2016. Improved drought tolerance by α-naphthaleneacetic acid-induced ROS accumulation in two soybean cultivars. *J. integrative agric*. 15(8): 1770-1784.

Xiong, L., Wang, R., Mao, G., and Koczan, J.M. 2006. Identification of drought tolerance determinants by genetic analysis of root response to drought stress and abscisic acid. *Plant Physiol*. 142: 1065–1074.

Xu, F., Wang, X.X.L., Wu, Q., Zhang, X., and Wang, L. 2012. Physiological responses differences of different genotype sesames to flooding stress. *Adv. J. Food Sci. Technol*. 4(6): 352-356.

Xu, K. and Mackill, D.1995. RAPD and RFLP mapping of a submergence tolerance locus in rice. *Rice Genet Newsl*, 12: 244-245.

Xu, K. and Mackill, D.J. 1996. A major locus for submergence tolerance mapped on rice chromosome 9. *Mol. Breeding* 2:219–224.

Xu, K., Xu, X., Fukao, T., Canlas, P., Maghirang-Rodriguez, R., and Heuer, S. 2006. *Sub1A* is an ethylene-response-factor-like gene that confers submergence to tolerance to rice. *Nat. Letters.* 442:705–708.

Yan, B., Dai, Q., Liu, X., Huang, S., and Wang, Z., 1996. Flooding-induced membrane damage, lipid oxidation and activated oxygen generation in corn leaves. *Plant and soil*. 179(2):261-268.

Yin, D.M., Chen, S.M., Chen, F. D., Guan, Z. Y. Fang, W. M. 2010.Morpho-anatomical and physiological responses of two Dendranthema species to waterlogging. *Environ. Exp. Bot*. 68:122–130.

Younis, M. A., Stickler, F. C., and Sorensen, E. L. 1963. Reactions of seven alfalfa varieties under simulated moisture stresses in the seedling stage. *Agron. J.* 55:177-181.

Yucel, D.O., Anlarsal, A.E., Mart, D., and Yucel, C.2010. Effect of drought stress on early seedling growth of chickpea (*Cicer arientinum* L.) genotypes. *World Appl. Sci. J.* 11(4):478-485.

Zhang, J. and Zhang, X. 1994. Account for much of the ABA accumulation in flooded pea plants. *J. Exp. Bot.* 45: 1335-1342.

Zhang, X., Shabala, S., Koutoulis, A., Shabala, L., Johnson, P., and Hayes, D. 2015.Waterlogging tolerance in barley is associated with faster aerenchyma formation in adventitious roots. *Plant Soil* 394:355–372.

Zhang, Y. X., Wang, L. H., Li, D. H., Yuan, G., Lu, H. X., and Zhang, X. R. 2014. Mapping of sesame waterlogging tolerance QTL and identification of excellent waterlogging tolerant germplasm. *Agric. Sci. China* 47(3): 422-430.

Zhou, M. Z. 2014. A comparison of screening methods to identify waterlogging tolerance in the field in *Brassica napus* L. during plant ontogeny. *.Agric. Sci. Res.* 2(2): 325-327.

Zhou, W., Zhao, D., and Lin, X. 1997. Effects of waterlogging on nitrogen accumulation and alleviation of waterlogging damage by application of nitrogen fertilizer and mixtalol in winter rape (*Brassica napus* L.). *J. Plant Growth Reg*. 16: 47–53.

Zhu, X., Shi, H., Li, X., and Jin, S.2020. Salicylic acid induces physiological and biochemical changes in peony under waterlogging stress. *Acta Sci. Pol. Hortoru.* 19(1):41-52.

Appendix

Appendix-1

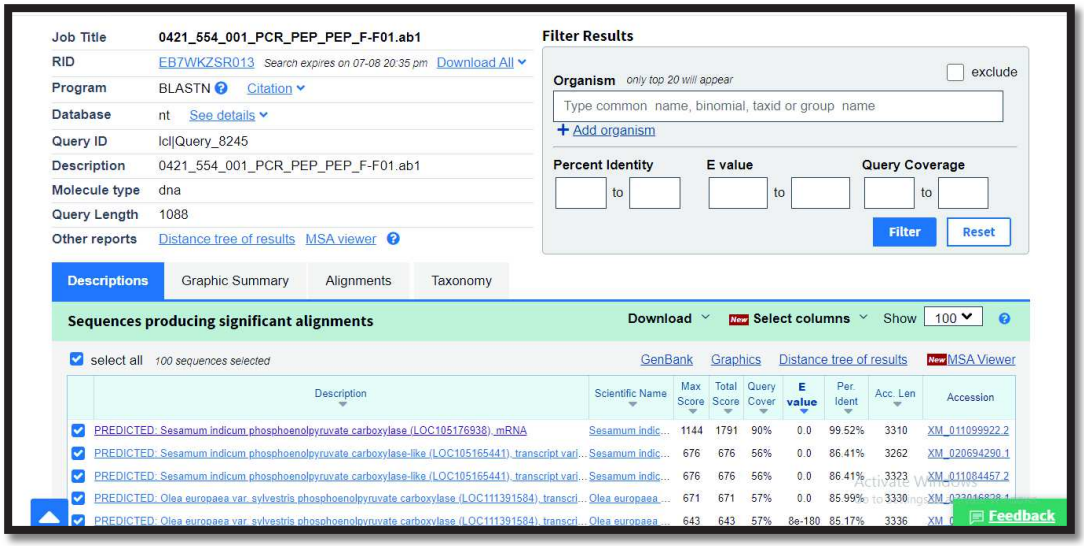
Phosphoenol pyruvate carboxylase **gene from sesame genotypes**

1. *Phosphoenol pyruvate carboxylase* gene from sesame var. Thilarani

>0421_554_001_PCR_PEP_E PEP_F-F01.ab1

ACCGTTATGGCGTAAGGCACCATCTGATGTCTTAGCAGTCGAGCTTCTA
CAACGTGAATGCCATGTGAAGCAGCCATTAAGAGTCGTTCCGCTCTTTG
AGAAACTAGCTGATCTCGAGGCTGCCCCTGCTGCTGTTGCACGCCTTTT
CTCGATCGATTGGTACAAAAGCCGGATCAACGGGAAGCAAGAAGTCAT
GATCGGGTACTCCGATTCTGGTAAAGATGCTGGTAGGCTGTCAGCAGCA
TGGCAATTGTACAAAGCTCAGGAGGAGCTTATCAAAGTTGCAAAGGAA
CATGGCGTGAAACTGACGATGTTCCACGGCCGAGGCGGAACTGTCGGA
AGAGGAGGTGGCCCCACTCACTTGGCTATATTGTCTCAACCACCAGAAA
CTATCCATGGATCTCTCCGTGTTACAGTTCAGGGAGAAGTTATTGAACA
ATCATTCGGTGAGGAGCATTTGTGCTTCAGGACGCTCCAGCGTTTCACT
GCTGCTACACTAGAACACGGAATGCATCCACCTGTCTCCCCCAAACCAG
AATGGAGGGCACTGCTGGATGAAATTGCTGTTGTTGCCACCGAGGAGT
ACCGGTCGATTGTCTTCAAAGAACCCCGGTTTGTCGAGTATTTCCGCCT
AGTAAGTGACTATAGATATTGTATTACATTTATTTATTATCTCCAAGTAT
TATCAATTGTCATGACAATTCTTTTGTATTCTTAGGCCTCGCCGGAATTG
GAATATGGTAGGATGAACATCGGCAGTCGTCCTTCAAAACGTAAACCT
AGTGGCGGGATCGAATCACTAAGAGCCATTCCTTGGATCTTCGCCTGGA
CTCAGACCCGATTTCATCTCCCCGTTTGGCTAGGCTTTGGAGGAGCATT
CAAATATGCCATAGGAAAAGATATCAAGAACCTGAAAATGCTGCAAGA
AATGTAAAATGAATGGCCTTTCTTCAGAGTCACGATTGACTTAGTCGAG
ATGGTTTTCGCCAAGGGAGACCCCGGCATTGCTGCATTGTATGACAAAC
TCCTAGTGTCGGAAGACTTGTGGTCGTTTGGCGAGCGATTGAGGGCACA
TTTGAAGGAAAAGA

Sequence similarity search using BLASTn revealed 99.52 % identity to PREDICTED: *Sesamum indicum phosphoenolpyruvate carboxylase* (LOC105176938)



Sequence similarity of *Phosphoenol pyruvate carboxylase* gene from sesame var. Thilarani identified using BLASTn

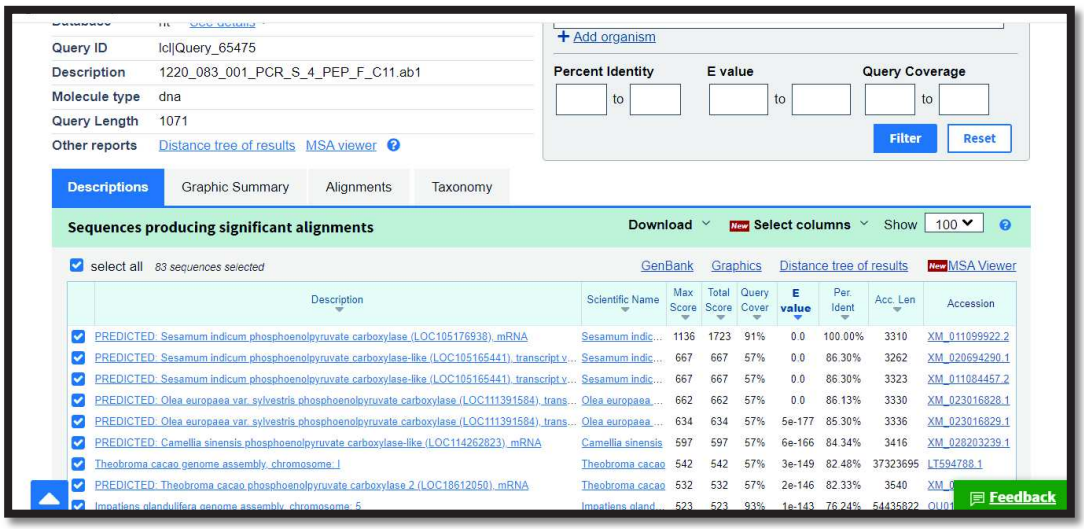
2. *Phosphoenol pyruvate carboxylase* gene from sesame var. Kayamkulam 1

>1220_083_001_PCR_S_4_PEP_F_C11.ab1

ACACATCTGATGTCTTAGCAGTCGAGCTTCTACAACGTGAATGCCATGT
GAAGCAGCCATTAAGAGTCGTTCCGCTCTTTGAGAAACTAGCTGATCTC
GAGGCTGCCCCTGCTGCTGTTGCACGCCTTTTCTCGATCGATTGGTACA
AAAGCCGGATCAACGGGAAGCAAGAAGTCATGATCGGGTACTCCGATT
CTGGTAAAGATGCTGGTAGGCTGTCAGCAGCATGGCAATTGTACAAAG
CTCAGGAGGAGCTTATCAAAGTTGCAAAGGAACATGGCGTGAAACTGA
CGATGTTCCACGGCCGAGGCGGAACTGTCGGAAGAGGAGGTGGCCCCA
CTCACTTGGCTATATTGTCTCAACCACCAGAAACTATCCATGGATCTCT
CCGTGTTACAGTTCAGGGAGAAGTTATTGAACAATCATTCGGTGAGGA

GCATTTGTGCTTCAGGACGCTCCAGCGTTTCACTGCTGCTACACTAGAA
CACGGAATGCATCCACCTGTCTCCCCCAAACCAGAATGGAGGGCACTG
CTGGATGAAATTGCTGTTGTTGCCACCGAGGAGTACCGGTCGATTGTCT
TCAAAGAACCCCGGTTTGTCGAGTATTTCCGCCTAGTAAGTGACTATAG
ATATTGTATTACATTTATTTATTATCTCCAAGTATTATCAATTGTCATGA
CAATTCTTTTGTATTCTTAGGCCTCGCCGGAATTGGAATATGGTAGGAT
GAACATCGGCAGTCGTCCTTCATAACGTAAACCTAGTGGCGGGATCGA
ATCACTAAGAGCCATTCCTTGGATCTTCGCCTGGACTCAGACCCGATTT
CATCTCCCCGTTTGGCTAGGCTTTGGAGGAGCATTCAAATATGCCATAG
GAAAAGATATCAAGAACCTGAAAATGCTGCAAGAAATGTATAATGAAT
GGCCTTTCTTCATAGTCACGATTGACTTAGTCGAGATGGTTTTCGCTAG
GGAGACCCGGCATTGCTGCAATGTATGACAACTCCTAATGTCGAAGACT
TGTGATCCTTTGGCGAGCGAATGAGGGCACCTATGGAGAAAAAAAAGA
A

Sequence similarity search using BLASTn revealed 100% identity to PREDICTED: *Sesamum indicum phosphoenolpyruvate carboxylase* (LOC105176938)



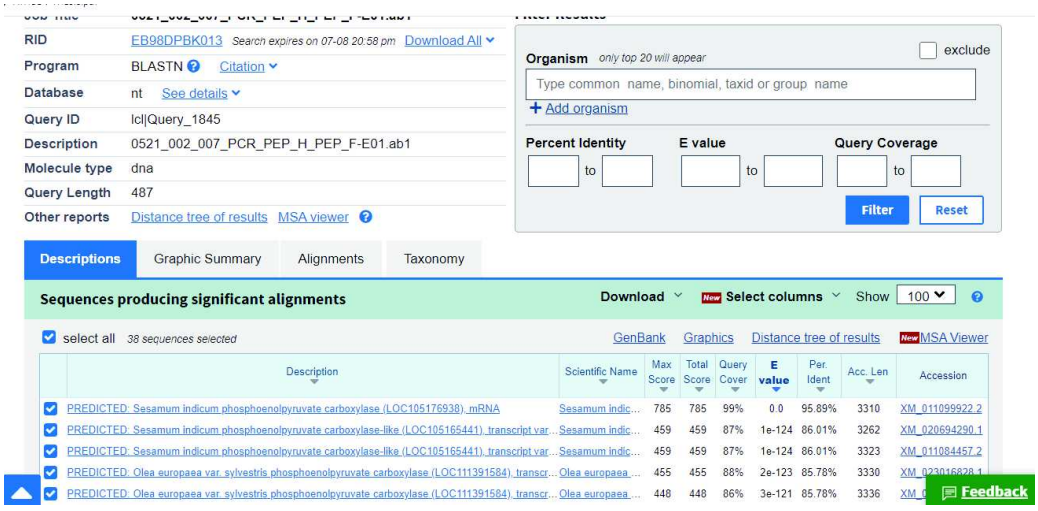
Sequence similarity of *Phosphoenol pyruvate carboxylase* gene of sesame var. Kayamkulam 1 identified through BLASTn

3. *Phosphoenol pyruvate carboxylase* gene from Thilatara

>0521_002_007_PCR_PEP_H_PEP_F-E01.ab1

ACATTTTATGGCGAACGCACCATCTGATGTCTTAGCAGTCGAGCTTCTA
CAACGTGAATGCCATGTGACGCATCCATTAAGAGTCGTTCCGCTCTTTG
AGAAACTAGCTGATCTCGAGGCTGCCCCTGCTGCTGTTGCACGCCTTTT
CTCGATCGATTGGTACAAAAGCCGGATCAACGGGAAGCAAGAAGTCAT
GATCGGGTACTCCGATTCTGGTAAAGATGCTGGTAGGCTGTCAGCAGCA
TGGCAATTGTACAAAGCTCAGGAGGAGCTTATCAAAGTTGCAAAGGAA
CATGGCGTGAAACTGACGATGTTCCACGGCCGAGGCGGAACTGTCGGA
AGAGGAGGTGGCCCCACTCACTTGGCTATATTGTCTCAACCACCAGAAA
CTATCCATGGATCTCTCCGTGTTACAGTTCAGGGAGAAGTTAATGAATC
TTTTTTTGTGAGTAGAATTTGAGCATCAGGACGCTCCACTGCGTTTCAG

Blastn result revealed 95.89 % identity to PREDICTED: *Sesamum indicum phosphoenolpyruvate carboxylase* (LOC105176938)



Sequence similarity of *Phosphoenol pyruvate carboxylase* gene from sesame var. Thilatara identified through BLASTn

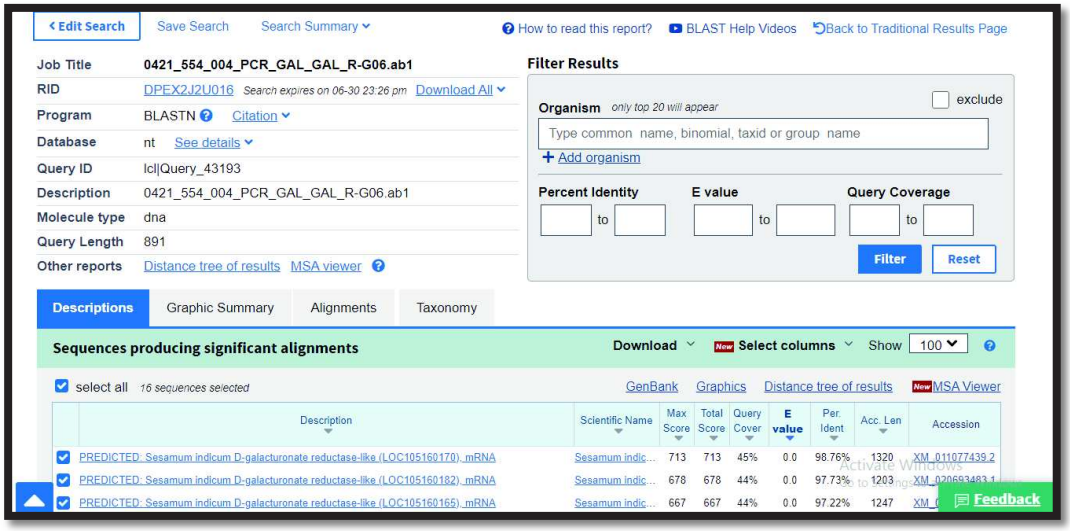
Galacturonate reductase **gene from sesame genotypes**

1. *Galacturonate reductase* gene from sesame var. Thilatara

>0421_554_004_PCR_GAL H GAL_R-G06.ab1

TAAGAACGGCATTAACCTCGTGCATCAAGCTCCATAAACAAGATCATG
GGGTCCGAGAATAGACGCCAAAGTTACCCCTTTTCGCTGCAGGAGTTGG
TCAATCTTTTCAAGATCATCGGTACTTAGCGCCCAGTCGAATATTTGAA
GATTTTCTCTCATCCTCTGCTTGTTGAAGCTCTTTGTGACAATGCTGACT
CCTTGCTCATACGCCCATCGCAGCGCCACCTGCGCAGTTGTCTTCCCCTT
GGCCTTGGCAATGTCTGCTAGCACATCACTTTCTACAATTCTGTTGTCTC
CCCATTTAGTGTTGTTTGCTCCCAAGGGAGAGTAGGCAGTAACGTGAAC
GCCTCTTGCCTTGCAGAACTCTCTCAATTGCTTTTGCTGCCAAAGAGGG
TTCATCTCCACCTATATACAACATGGAAAGATTCAAGAAGTGTTCTTGT
GCTGCCCGAAAAATATACCATTGTGGATATTGTGTGATTTATATTTAAA
TTATTTCAAAAACAGTCTCAAATAAACAAGTCTCCCTCCAGAAAATCAT
TGTAATGTGTAAAGTAACATAATAAATTGAATAAAGCTTGACATTTACT
AATAGAAAGCCAATACAAATGCCATTGTCCGTAAACCATGATTGACCTC
GTGTAGATATTAATGCTCATGACTTCAGAATAAAATCTGGTTACCCCAC
AATATCACCTCATTCCATTTGCAAGACCATAGAAAAAATTAGAACTCAA
TCTTTAATCATATTTCATAAACTCTAAAATACTTTTTGAGTAGATAATAT
ATTTCTCCCATCTAGGGATATAAACGAGTCGAGCTCGACCTTTTTTGGC
GAGTTCGAGCTTGAGCTCAAATTATTGTGTAGAAGAAAAAATAAAAAA
AGAAAAT

BLASTn had shown 98.76 % identity to PREDICTED: *Sesamum indicum* D-galacturonate reductase-like (LOC105160170), mRNA



Sequence similarity of *Galacturonate reductase* gene from sesame var. Thilatara identified through BLASTn

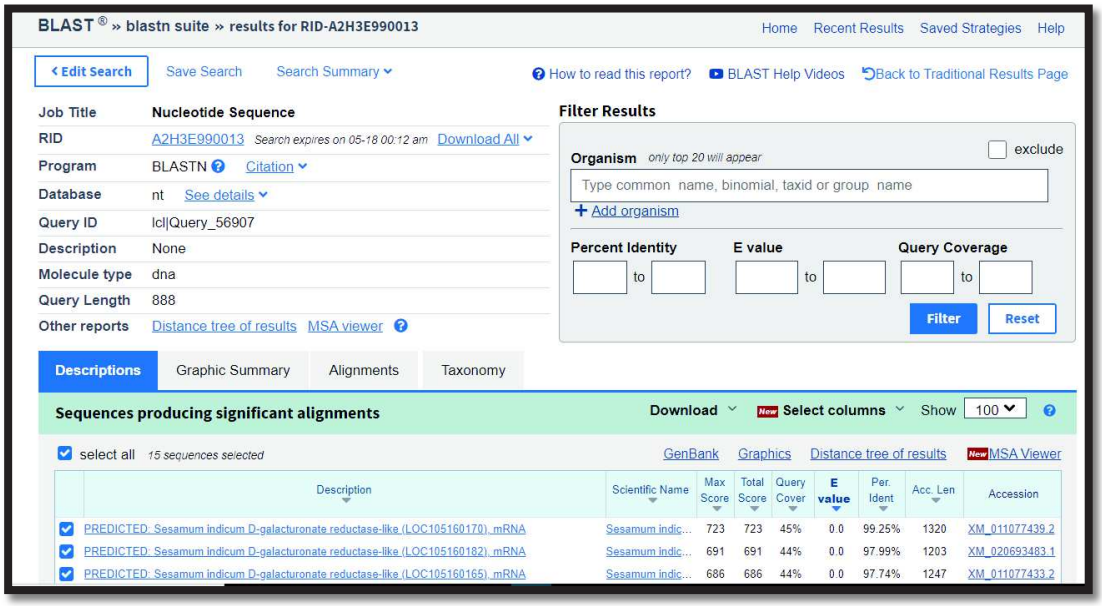
2. *Galacturonate reductase* gene from *S. malabaricum*

>0521_002_022_PCR_GAL_A_GAL_R-D03.ab1

AGAGAAGGAACACTGCAATTAACCTCGGCATCAAGCTCCAAAACAAGA
TCATGGGGTCCGAAGAATAGACGCCAAAGTTACCCCTTTTCGCTGCAGG
AGTTGGTCAATCTTTTCAAGATCATCGGTACTTAGCGCCCAGTCGAATA
TTTGAAGATTTTCTCTCATCCTCTGCTTGTTGAAGCTCTTTGTGACAATG
CTGACTCCTTGCTCATACGCCCATCGCAGCGCCACCTGCGCAGTTGTCT
TCCCCTTGGCCTTGGCAATGTCTGCTAGCACATCACTTTCTACAATTCTG
TTGTCTCCCCATTTAGTGTTGTTTGCTCCCAAGGGAGAGTAGGCAGTAA
TGTGAACGCCTCTTGCCTTGCAGAACTCTCTCAATTGCTTTTGCTGCCAA
AGAGGGTTCATCTCCACCTATATACAACATGGAAAGATTCAAGAAGTG
TTCTTGTGCTGCCCGAAAAATATACCATTGTGGATATTGTGTGATTTATA
TTTAAATTATTTCAAAATCAGTCTCAAATAAACAAGTCTCCCTCCAGAA
AATCATTGTAATGTGTAAAGTAACATAATAAATTGAATAAAGCTTGACA
TTTACTAATAGAAAGCCAATACATATGCCATTGTCCCTAAACCATGATT
GACCTCGTGTAGATATTAATGCTCATGACTTTAGAATAAAATCTGGTTA
CCCCACAATATCACCTCATTCCATTTGCATGACCATAGAAAAAATTAGA

ACTCAATCTTTAATCATATTTCATAAACTCTAAAATACTTTTTGAGTATA
TAATATATTTCTCCCATCTAGGGATATAAACGAGACGAGCTCGACCTTT
TTTGTCTAGTTCGAGCTTGACCTCGATTTTGAGTTGAGGAGAAAAAAAA
AAA

BLASTn results have shown that 99.25 % identity to PREDICTED: *Sesamum indicum* D-galacturonate reductase-like (LOC105160170), mRNA



Sequence similarity of *Galacturonate reductase* gene from *S. malabaricum* identified through BLASTn

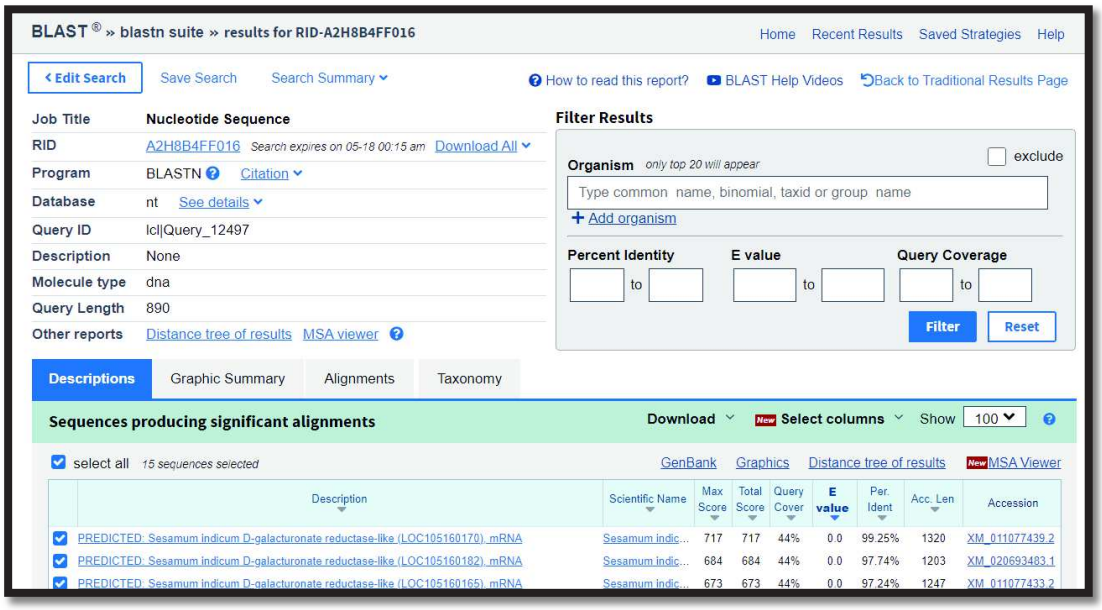
3. *Galacturonate reductase* gene from CO 1

>0521_002_024_PCR_GAL_F_GAL_R-F03.ab1

AGAGAAGACCGGCAATTAACCTCGGCATCAAGGCTCCAAAACAAGATC
ATGGGGGTCCGAGAATAGACGCCAAAGTTACCCCTTTTCGCTGCAGGA
GTTGGTCAATCTTTTCAAGATCATCGGTACTTAGCGCCCAGTCGAATAT
TTGAAGATTTTCTCTCATCCTCTGCTTGTTGAAGCTCTTTGTGACAATGC
TGACTCCTTGCTCATACGCCCATCGCAGCGCCACCTGCGCAGTTGTCTT
CCCCTTGGCCTTGGCAATGTCTGCTAGCACATCACTTTCTACAATTCTGT

TGTCTCCCCATTTAGTGTTGTTTGCTCCCAAGGGAGAGTAGGCAGTAAC
GTGAACGCCTCTTGCCTTGCAGAACTCTCTCAATTGCTTTTGCTGCCAAA
GAGGGTTCATCTCCACCTATATACAACATGGAAAGATTCAAGAAGTGTT
CTTGTGCTGCCCGAAAAATATACCATTGTGGATATTGTGTGATTTATATT
TAAATTATTTCAAAAACAGTCTCAAATAAACAAGTCTCCCTCCAGAAAA
TCATTGTAATGTGTAAAGTAACATAATAAATTGAATAAAGCTTGACATT
TACTAATAGAAAGCCAATACAAATGCCATTGTCCGTAAACCATGATTGA
CCTCGTGTAGATATTAATGCTCATGACTTCAGAATAAAATCTGGTTACC
CCACAATATCACCTCATTCCATTTGCAAGACCATAGAAAAAATTAGAAC
TCAATCTTTAATCATATTTCATAAACTCTAAAATACATTTTGAGTAGATA
ATATATTTCTCCCATCTACGGATATAAACTAGTCAAGCTCGACCTTTTTT
GTCTAGTTCGAGCTTGACCTCGATTTTGAGTTGCATGCCCAACAGAAAA
AAAT

BLASTn analysis had shown 99.25 % identity to PREDICTED: *Sesamum indicum* D-galacturonate reductase-like (LOC105160170), mRNA



Description of alignment result using BLASTn of *Galacturonate reductase* gene from CO 1

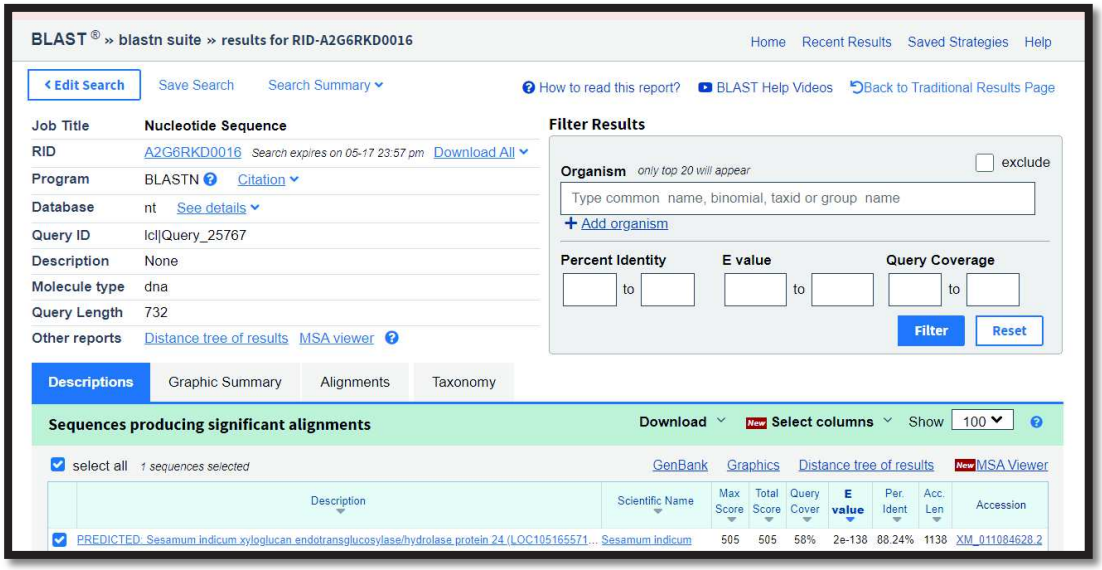
***Xyloglucan endotransglycosylase* gene from sesame genotypes**

1. *Xyloglucan endotransglycosylase* gene from *S. malabaricum*

>0521_002_014_PCR_XYLO_A_XYL_R-D02.ab1

CTTGCACCGACAGATCCCCGTACCAAGAGAAGAATACGAGTTATTAAA
ATCTGGAGCGCTTGCTCGCGGGTGGAAGGCCCTGAGGGAACCTGGGCA
TATCATCGCAGTAATTGTAGATCATATGCTTCTGGTGAACCCCTCTCCA
CCTGTTTCTGCCTTTGGAATAAAGTTCCTGGGTTTGCCATTCCTCGTTAC
TGAAACTATCTGCCGATGTAGATCCGCAGGATCCCCCTATCGACCCGCT
TACACACGCGTTGATCTTGAAATTCCTGTGTTATGCTACACATGGAGCC
TTGTGCCCCTCTGTTTTGACCCCCCCCCCCTGTGTTGCCCCCTCATCTGC
GCTCCACACGCTGCAGTGTGCTCTCACGGGGTGACTTGTGGGAGAGGG
GACACCCCCGCGTGTGTGTAATACTCTATTTGTAGTGTTTGTACAACGA
CTCTGAGATCGCGGGTACACGTTTTCTCTGGGGAACTCTCTATCGCGTC
TCGTTTAACGCTCTGAGATCTCTATATGAGATGTACTCTCGCGTCGCGT
GTATATGTGCAGCTACAAAGCACACAGAGGGAGTGTGCGTGTGCCGGA
TATCTAGATGACTCATCTCATATGAGAGAGCATTTAGAGAGGTGAGAC
AGATATCACGAGATGCTCAGGATCAGCACTACAACGTGTGGAGTCCGC
AGAACGCACGAACGAGACGAGTGCTGCTACAGTCCTGCAATACACTGT
GT

BLASTn had shown 88.24 % identity to PREDICTED: *Sesamum indicum* Xyloglucan endotransglucosylase/hydrolase protein 24 (LOC105165571), mRNA



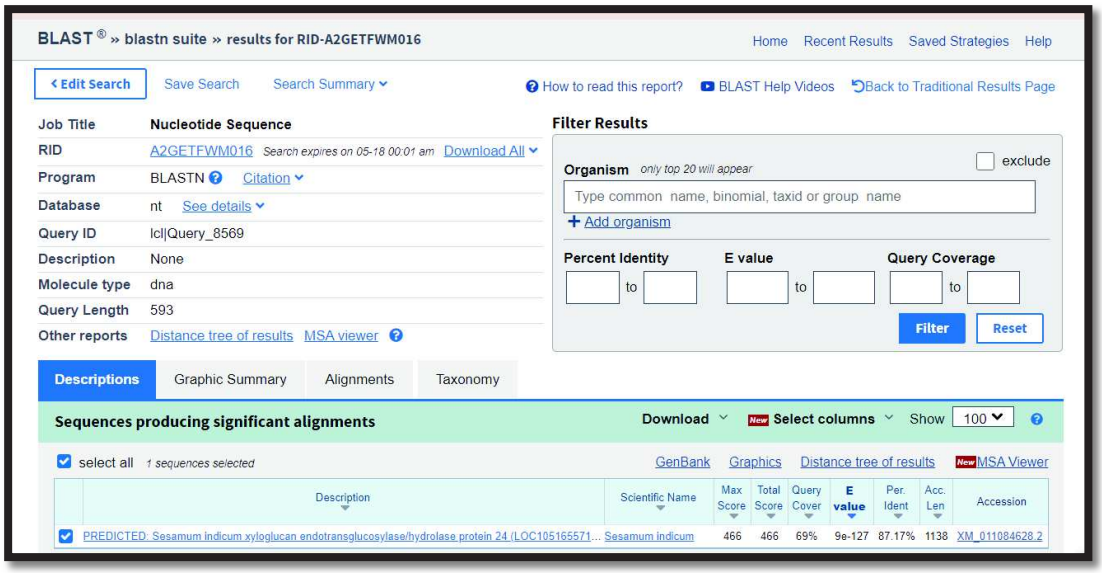
Sequence similarity of *Xyloglucan endotransglycosylase* gene from *S. malabaricum* identified through BLASTn

2. *Xyloglucan endotransglycosylase* gene from CO 1

>0521_002_016_PCR_XYLO_F_XYL_R-F02.ab1

TCGTGGCACTGACAGACCCCCATTACCAAGAGAAAGATACAGAGTTAT
TAAAATCTGGAGCGCTTGCTCGCGGGTGGAAGGCCCTGAGGGAACCTG
CGCATATCCTCACAGTAATTGTAGATCATATGCTTCTGGTGAACCCCTC
TCCACCTGTTTCTGCCTTTGTTATAAAGTTCCTGGGTTTGCCATTCCTCG
TTACTGAAACTATCTGCCGATGTATATCCCCAGGATCCCCCTATATACC
CGCTTACACACGCGTTGATCTTGAAATTCCTGTGTAATGCTACAAATGG
AGCCTTGTGCCCCTCTGTTTTGACCCCCCCCCCCTGTGTTGCCCCCACAT
CTGTGTTCCACACGGTGCGGTGTGCTCTCATGGGGTGACTTGTGGGAAA
GGGGACACCCCCGCGGGTGTGGAATACTCTAAATGTAGTGTTGTGCACC
CAGAGTATGAGATCATGTGGTATACGTTTTCTCAGGGAAAATTCTCTAT
CGCATCGCGGGTATACACTCTGAGAAAATTATGTGAGAAAAAATATAT
CGTCTCGTGAATTATGTGCAGTCTACAAAACCCCCCATGAGGGTGAGAG
TGCTTG

BLASTn had shown 87.17 % identity to PREDICTED: *Sesamum indicum* Xyloglucan endotransglucosylase/hydrolase protein 24 (LOC105165571), mRNA



Sequence similarity of *Xyloglucan endotransglycosylase* gene from sesame var. CO 1 identified through BLASTn

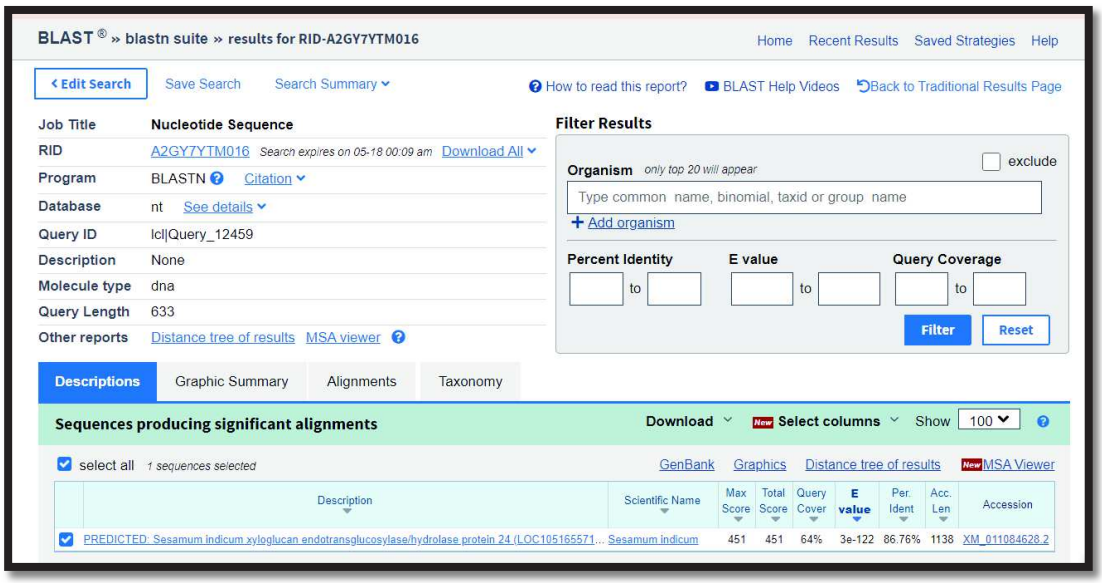
3. *Xyloglucan endotransglycosylase* gene from Thilatara

>0521_002_020_PCR_XYLO_H_XYL_R-B03.ab1

GCAAACGAGCACTGACAGACCCCGTACCAAGAGAAAGCTACAGAGTTA
TTAAAATCTGGAGCGCTCGCTCGCGGGTGGAAGGCCCTGAGGGAACCT
GGGGATATCCTCACAGTAATTGTAGATCATATGCTTCTGGTGAACCCCT
CTCCACCTGTTTCTGCCTTTGGAATAAAGTTCCTGGGTTTGCCATTCCTC
GTTACTGAAACTATCTGCCGATGTAGATCCGCAGGATCCCCCTATATAC
CCGCTTACACACGCGTTGATCTTGAAATTCCTGTGTAATGCTACAAATG
GAGCCTTGTGCCCCTCTGTTTTGACCCCCCCCCCCTGTGTTGCCCACACA
TCTGTGCTCCACACGCTGCAGTGTGCTCTCATGGGGTGAGATGTGGGAG
AGGGGACACCTCTGCGTGTGTGTAAAACTCTAAATGTAATGTGGTCTAC
AAAAAAACTGAAATCACGTGGTACAAGTTTTCTAAAAGAAAATACTAT

ATCTCAACTCGTTATACACTCTGAGATCACATATATGAGAAAACTATCT
TGTCGCGTGAATAAGTACAGTGTACACAAAAGCCCCACTAGAGAGTGT
GTGTGCGTGTGCTGCGAGTATTTATATTGAGTACTCTAACTTGTGGG

BLASTn had shown 86.76 % identity to PREDICTED: *Sesamum indicum* xyloglucan endotransglucosylase/hydrolase protein 24 (LOC105165571), mRNA



Sequence similarity of *Xyloglucan endotransglycosylase* gene from sesame var. Thilatara identified through BLASTn

Inositol oxigenase gene from sesame genotypes

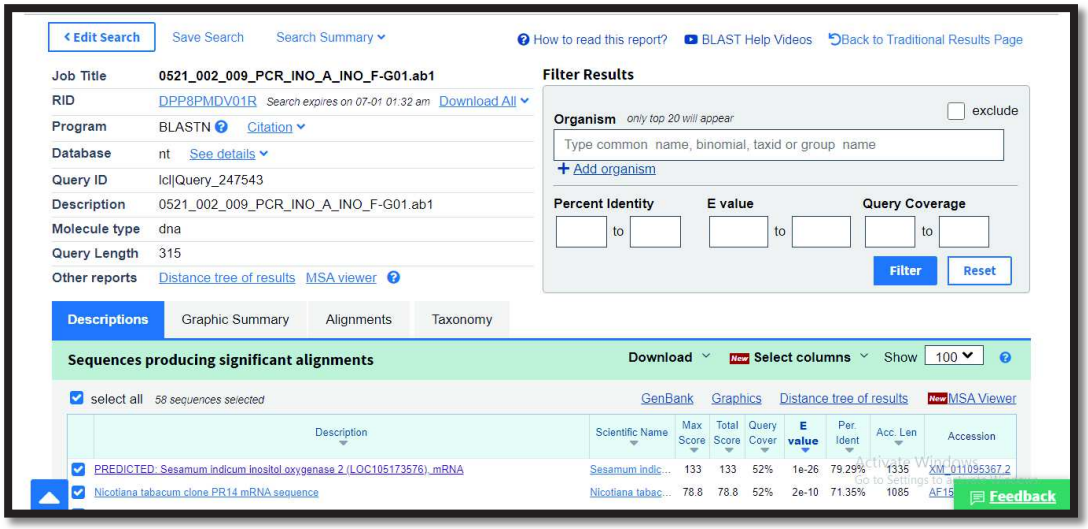
1. *Inositol oxigenase* gene from *S. malabaricum*

>0521_002_009_PCR_INO_A_INO_F-G01.ab1

GATTACCTTGGATAGAGGGGATATGAGCATTTCGCGATGATGCGAAAA
GCTGAACGATGTGGCATTGACAGCGAGCCTGATTTGAACCAACCATTGT
TGATTCTTGCTGCTGCAGCGGAGGCCATAAGAAATGACCCCCCCCATGA
GGATTGGCTTCATTTGGCTGCCCTCCTCCGTACCATCAAATATTATTCAA
TATACAAGGGGGTGTGATTATTGTTGGATATTATTATTTTTGTCCTCATT

TTTTGCATACAGACTGCATCATCGAAAAAGTCTGCTTTCTCCTACTATCT
TTGGATGGCTGCCCTTGTG

BLASTn had shown 79.29 % identity to PREDICTED: *Sesamum indicum* inositol oxygenase 2 (LOC105173576), mRNA



Sequence similarity of *Inositol oxigenase* gene from *S. malabaricum* identified through BLASTn

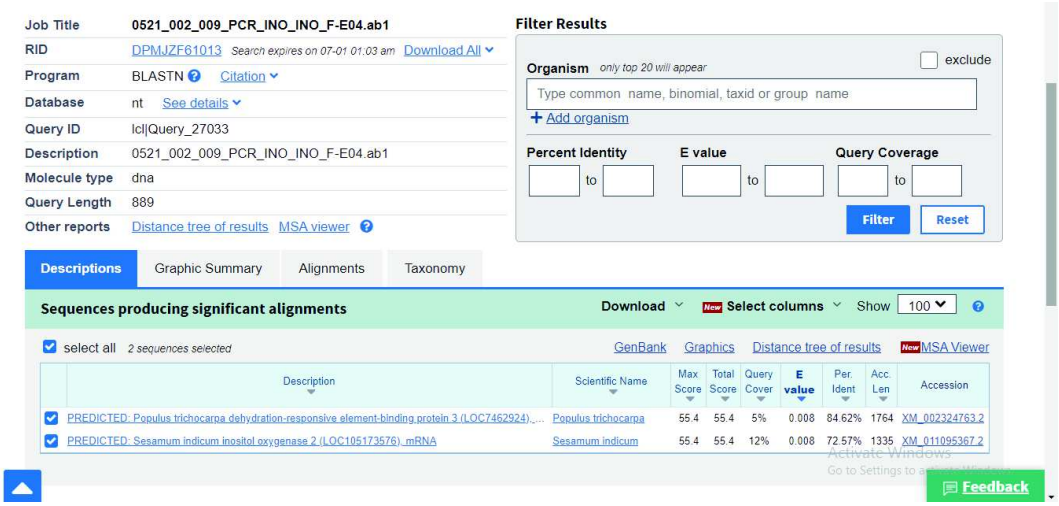
2. *Inositol oxigenase* gene from Kayamkulam 1

>0521_002_009_PCR_INO_D INO_F-E04.ab1

GACGGTTTGAATATATATGGATATGAGCATAGGGCGATGATTGCGAAA
AGCTGAGGATGAGGACACTGACACCCAGCTTGATTTGAACCAACCATT
GTTGTTCCCTGCTGCGGCAGAGGCCGCCAAAAGAAATTACCCCCCCCAG
GATGGTTGGCTTTTTTTGCCCCCCCTCCTCGGTATTATCAAATGTTCTAT
AAACGAAAGGGTTTGGATGATTGGGATTATCCATAATTTGAACATTCTT
TTTTTGCAGAGGGGCCAAATATCGAAAAAAAGTGCTTCTTCCTCCTTTT
TGGGGGGGGCCCCCCGGGGGGGGTGTTGGGGGAGATACCCCCCCTCGT
GGGTGTTTTTTTGAAGCCTCCTGTGCCCCCCAGGGGGAATATTAAAAAA
GGGCGCCAAAAAATATAGCTTTTTTTTTAGATATACCCCCTTTGTTGGTA
AAAAATAATTAAAAAATGTTTTGTGTTTTGGGGAAAAACAATATATCTG

CGCGCTTTTTTCCCCACCTCGGAGATTCCTCCCACACACACCCGTCTGCA
TACAACACCAAGGGTGTTATTTAAAAAGGGGATGTCGTATAGAGGTTG
TTCGCGTGGCGGGGGGCGCACGAACACTATATGCTGGGTGGTTTTTTTC
ACATAAAAAAAAAGGGGGGTGATTTAGTGTTTTTTTTTTCTTTTGTGCC
ACCCAAAATCCCCCCCAAACAGTTGGGTGAATTCACCCTCGGGGGGGG
GAGAAAAAAAAAGGGAAACCACTCCACCCCCCGGCGCGGGTTTGTTTA
ACCGTCTCCCCCCCCTTCTCTCTCCCGAAAAAATGTGAGAGATTCTTTAT
AATTATATTATCTCTCTGGAAAAAAAGAGGTGTTTTTTTCCCTCTCTTGA
AAGAAAAA

BLASTn had shown 72.57 % identity to PREDICTED: *Sesamum indicum* inositol oxygenase 2 (LOC105173576), mRNA



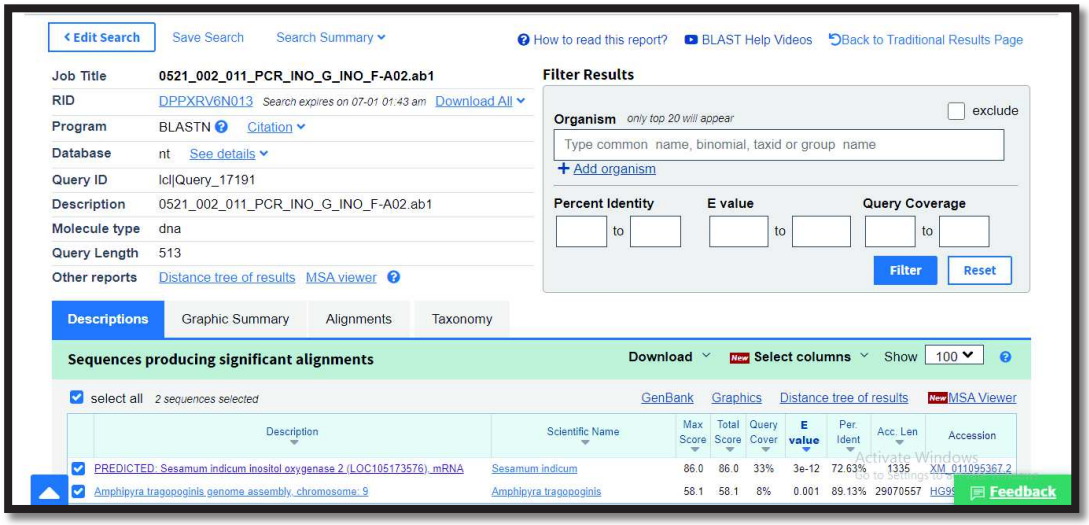
Sequence similarity of *Inositol oxigenase* gene from Kayamkulam 1 identified through BLASTn

3. *Inositol oxigenase* gene from Ayali

>0521_002_011_PCR_INO_G_INO_F-A02.ab1

TAAATTAAAACTTGATAGAGTGGATTTGAGCATTTGCGGTGCTGCGAAA
AGCTGAGGATGAGACCCCTGACACCGATCTTGATTTGAACCAACCCTTG
TTGTTTCTTGCTGCTGCAGAGGACGCCAAAAGAAATTATCCCCCCCATG
ATGGTTGGCTTTTTTTGGCCCCCCTCCTCCGTCTTATAATATGTTCTATT
ATATTGAGGGGTTGGGATTATAGGTGGATACCTAATTTGAAACCTTATT
TTGCATAGGGGGACTGCATATCGTAAAGGGTGCTTCTTCCTCCTTTTTTT
GGGGGGCCCCCCGGGGGGGGGTGGTGGGGGAGATACCCCCCCTCGTGGG
TGTGTTTTTGATGCCTCTTTTGTCCACCAGGGAAAAAATTAATAAAAGG
CCACCAAAAATATAGCTTTTTTTCGTGATAAACCCTTCGTGTGGGTGAA
ATAATATTAAAAAATGTGTTTTGTTTTTGGGTATAACAACATATATCCG
TGCTTTTTTTATTATTTGGAAA

BLASTn had shown 72.63 % identity to PREDICTED: *Sesamum indicum* Inositol oxygenase 2 protein (LOC105173576), mRNA



Sequence similarity of *Inositol oxigenase* gene from Ayali identified through BLASTn

Abstract

EVALUATION OF SESAME GENOTYPES FOR TOLERANCE TO WATER LOGGING AND DEVELOPMENT OF MITIGATION STRATEGIES

By

SREEPRIYA S

(2017-21-020)

ABSTRACT OF THE THESIS

Submitted in partial fulfillment of the requirement for the degree of

Doctor of Philosophy in Agriculture

(Plant Physiology)

Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF PLANT PHYSIOLOGY

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2021

Abstract

The present study was initiated to identify constraints of sesame farmers in Kerala, to screen sesame genotypes for tolerance to waterlogging, identification of suitable ameliorative treatment for waterproofing sesame, molecular characterization of sesame genotypes with genes and markers reported for waterlogging tolerance and screening for drought tolerance.

Sesame growing farmers were surveyed in three districts of Kerala *viz*., Alappuzha, Kollam and Thrissur during 2018. Data was analyzed using Garrett ranking technique. Identified constraints were high labour cost, excessive rain fall, drought, weed infestation, unavailability of labour, pest and disease, marketing problem, problems for transportation, drying and threshing, and storage problems in decreasing order of their Garrett rank.

Screening for waterlogging tolerance was conducted at College of Agriculture, Vellanikkara during 2018 with 15 sesame genotypes. Varietal influence on waterlogging at vegetative (20 DAS) and flowering stage (40 DAS) for a period of 72 hr were studied separately. Sesame genotypes are found to be sensitive to waterlogging at vegetative stage than flowering stage. Waterlogging for 72 hrs affected normal growth of sesame plants in terms of reduced plant height, shoot dry weight, reduced chlorophyll content, increased lipid peroxidation, reduced nitrate reductase enzyme activity and reduced soluble protein content. The wild genotype, *Sesamum malabaricum* possessed 100% survival whereas genotype CO 1 (30.50 %) recorded the lowest. Formation of adventitious roots located close to the surface was observed in tolerant genotypes, which can provided better access to oxygen. Root number was found to increase in all genotypes except susceptible ones such as CO 1, Thilatara and AT231. Using cluster analysis the 15 genotypes were grouped into 4 clusters as, Waterlogging tolerant (GT 10, TMV 7, Kayamkulam 1, Thilak, SVPR1, *S. malabaricum*, TMV5, Thilarani, Ayali,), Waterlogging susceptible (Thilatara, CO 1, AT 231), Moderately susceptible (TMV 3, TMV 4) and Moderately tolerant genotype (TMV6).

To identify the best ameliorant for water proofing sesame in the field a preliminary pot culture study was conducted at College of Agriculture, Vellanikkara during 2019, using sesame var. Thilak. Urea, KNO_3, *Pseudomonas fluorescens*, $Ca(NO_3)_2$, NAA, Salicylic acid and Tricyclazole were the treatments tried. Foliar application of KNO_3 ($5gL^{-1}$), Salicylic acid (100 ppm) and seed treatment (talc formulation 10g L^{-1}) and foliar application (culture broth 30mL L^{-1}) of *P. fluorescens* were found to be the most promising treatments in term of improved morphological, biochemical and physiological parameters.

Later a field study was conducted in a farmer's field at Alapuzha during February- May in 2019 with selected ameliorants. Results of the study showed enhanced production of branches, capsules per plant and seeds per capsules compared to untreated waterlogged control. Yield reduction due to waterlogging was minimized by the application of *P. fluorescens*, KNO_3 and salicylic acid which recorded 16.52, 13.73 and 7.93 per cent improvement in yield respectively as compared to waterlogged control. The result indicated that water proofing sesame var. Thilak with ameliorants could improve yield by 8 to 17 per cent as compared to waterlogged control.

A lab study was conducted at College of Agriculture, Vellanikkara in 2020 to screen sesame genotypes for tolerance to PEG (Poly ethylene glycol) induced drought stress at seed germination stage. PEG induced drought affected seedling characters such as per cent of normal seedlings, shoot length, root length and vigour index. PEG concentration increased the per cent of abnormal seedlings and dead seedlings. Among the genotypes under investigation, higher stress tolerance index was recorded in *Sesamum malabaricum* (35), TMV3 (32), Ayali (32), Kayamkulam 1 (31) and Thilatara (27). The varieties CO1 (7), TMV5 (11), TMV7 (14) and Thilak (15) recorded the lowest stress tolerance index.

Z distribution analysis classified sesame genotypes into 6 groups as, Tolerant to both stress (*S. malabaricum*), Susceptible to both stress (CO 1), Moderately susceptible to both stress (AT231), Moderately tolerant to both stress (Ayali, Thilarani, Kayamkulam1, SVPR1), Tolerant to waterlogging but susceptible

to drought (TMV5, TMV 7) and.Tolerant to drought but susceptible to waterlogging (Thilatara).

Molecular characterization of selected sesame genotypes was done. Genes reported to be up regulated under waterlogging *viz*., *Phosphoenol pyruvate carboxylase*, *Inositol oxigenase*, *Xyloglucan endotransglycosylase* and *Galacturonate reductase* and QTL associated SSR markers (ZM 428, ZM 22) were studied. No polymorphism in bands of SSR markers were obtained. Sequencing of *Phosphoenol pyruvate carboxylase* gene revealed single nucleotide polymorphisms in susceptible genotype, Thilatara as compared to the moderately tolerant genotypes. Insertion of single nucleotide in *Xyloglucan endotransglycosylase* gene resulted in truncation of aminoacid sequence in translated protein sequence of susceptible genotype CO 1 compared to tolerant *S. malabaricum*.

The genotypes *S. malabaricum*, Ayali, Thilarani and Kayamkulam 1 performed better under both stress conditions can be incorporated in crop improvement programmes. In the field, as a water proofing technique application of *P. fluorescens* as seed priming of talc formulation (10 g L^{-1}) and foliar spray of culture broth (30 mL L^{-1}) or spraying KNO_3 (5g L^{-1}) or Salicylic acid (100ppm) can be suggested to reduce the growth inhibitory effect of waterlogging when unexpected heavy rains are forecasted during summer when the crop is normally grown.

സംഗ്രഹം

കേരളത്തിലെ എള്ളുകർഷകരുടെ പ്രശ്നങ്ങൾ മനസ്സിലാക്കുക, വെള്ളക്കെട്ടിനെ അതിജീവിയ്ക്കുവാൻ കഴിവുള്ള എള്ളിനങ്ങളെ കണ്ടെത്തുക, വെള്ളക്കെട്ട് മൂലം എള്ളിന്റെ വളർച്ചയിൽ ഉണ്ടാകുന്ന പ്രശ്നങ്ങളെ മറികടക്കാനുള്ള മാർഗ്ഗങ്ങൾ കണ്ടെത്തുക, വെള്ളക്കെട്ടിനെ അതിജീവിക്കാൻ പ്രാപ്തമാക്കുന്ന ജീനുകൾ തിരഞ്ഞെടുക്കപ്പെട്ട എള്ളിനങ്ങളിൽ ഉണ്ടോയെന്ന് കണ്ടെത്തുക, വരൾച്ചയെ ചെറുക്കാൻ പ്രാപ്തമായ എള്ളിനങ്ങളെ തിരഞ്ഞെടുക്കുക എന്നീ ലക്ഷ്യങ്ങളോടുകൂടിയാണ് കാർഷിക കോളേജ്, വെള്ളാനിക്കരയിൽ പഠനം ആരംഭിച്ചത്.

കേരളത്തിലെ 3 ജില്ലകളിലായി 90 എള്ളു കൃഷി ചെയ്യുന്ന കർഷകരെ സർവ്വേ നടത്തുകയുണ്ടായി. അമിതമായ തൊഴിൽ ചെലവ്, അമിതമായ മഴ, വരൾച്ച, കളകൾ, തൊഴിലാളികയുടെ അഭാവം, കീടങ്ങളും അസുഖങ്ങളും, വിപ്പനയുമായി ബന്ധപ്പെട്ട പ്രശ്നം, ഗതാഗതത്തിനും ചെടി ഉണക്കുന്നതിനും, എള്ളു വേർതിരിക്കുന്നതിനും ഉള്ള പ്രശ്നങ്ങൽ, സംഭരിക്കുബോൾ വരുന്ന പ്രശ്നങ്ങൽ എന്നിവയാണ് എള്ളു കർഷകരുടെ പ്രശ്നങ്ങൽ ആയി കണ്ടെത്തിയത്.

വെള്ളക്കെട്ടിനെ അതിജീവിക്കുന്ന എള്ളിനങ്ങളെ തിരഞ്ഞെടുക്കുന്നതിനായി വിവിധ ഗവേഷണകേന്ദ്രങ്ങളിൽ നിന്നായി 15 തരം എള്ളിനങ്ങൾ ശേഖരിച്ചു. ചട്ടികളിൽ നടത്തിയ പരീക്ഷണത്തിൽ 20 ദിവസവും, 40 ദിവസവും വളർച്ചയെത്തിയ ചെടികളെ പ്രത്യേകമായി 72 മണിക്കൂർ വെള്ളം കെട്ടി നിറുത്തി. 20 ദിവസം പ്രായമായ ചെടികളുടെ വളർച്ചയെയും അതിജീവനത്തേയുമാണ് വെള്ളക്കെട്ട് കൂടുതലായി ബാധിച്ചത്. ചെടിയുടെ ഉയരം, ചെടി ഉണങ്ങിയ ഭാരം, ഹരിതകത്തിന്റെ അളവ്, നൈട്രേറ്റ് റിഡക്ട്റ്റേസ് എൻസൈം, പ്രോട്ടീൻ എന്നിവയുടെ അളവ് കുറയുന്നതായും മെലൺഡൈആൾഡിഹൈഡിന്റെ അളവ് കൂടുന്നതായും കണ്ടെത്തി. സെസാമം മലബാറിക്കം എന്ന വന്യയിനം എള്ള് വെള്ളക്കെട്ടിനെ 100 % അതിജീവിച്ചു. C01 എന്ന എള്ളിനമാണ് വെള്ളക്കെട്ടിനെ ഏറ്റവും കുറവ് അതിജീവിച്ചത്. വെള്ളം കെട്ടിനിർത്തുമ്പോൾ എള്ള് ചെടികളിൽ ഉണ്ടായ അഡ്വെന്റിഷ്യസ് വേരുകൾ അന്തരീക്ഷത്തിലുള്ള ഓക്സിജൻ ലഭിക്കുന്നതിന് സഹായിക്കുന്നു. C01, തിലതാര, AT231 എന്നീ എള്ളിനങ്ങളിൽ ഒഴികെ മറ്റെല്ലാ ഇനങ്ങളിലും വേരുകളുടെ എണ്ണം കൂടുന്നതായി കാണുവാൻ സാധിച്ചു. ക്ലസ്റ്റർ അനാലിസിസ് വഴി 15 തരം എള്ളിനങ്ങളെ 4 ഗ്രൂപ്പുകളാക്കി തരം തിരിച്ചു.

1. വെള്ളക്കെട്ടിനെ അതിജീവിക്കാൻ കഴിവുള്ളവ- GT10, TMV7, കായംകുളം1, തിലക്, SVPR1, സെസാമം മലബാറികം, TMV5, തിലറാണി, അയാളി.
2. വെള്ളക്കെട്ടിനെ തീരെ അതിജീവിക്കാൻ കഴിവില്ലാത്തവ- തിലതാര, C01, AT231.
3. വെള്ളക്കെട്ടിനെ മിതമായ രീതിയിൽ അതിജീവിക്കാൻ കഴിവുള്ളവ- TMV6.
4. വെള്ളക്കെട്ടിനെ കുറച്ചു മാത്രം അതിജീവിക്കുന്നവ- TMV3, TMV4.

വെള്ളക്കെട്ട് മൂലം എള്ളു ചെടിയിൽ ഉണ്ടാകുന്ന വളർച്ചക്കുറവിനെ അതിജീവിക്കുന്നതിനായി ചില രാസവളങ്ങളും ഹോർമോണുകളും സ്യൂഡോമോണാസ് ഫ്ലൂറസെൻസ് എന്ന മിത്ര ബാക്ടീരിയയും ആണ് പരീക്ഷിച്ചത്. തിലക് എന്ന എള്ളിനത്തിൽ ചട്ടികളിൽ പരീക്ഷണം നടത്തി. 20 ദിവസം പ്രായമായ ചെടികളിൽ 72 മണിക്കൂർ വെള്ളം കെട്ടി നിർത്തി. പൊട്ടാസിയം നൈട്രേറ്റ് (5g/L), സാലിസിലിക് ആസിഡ് (100ppm),സ്യൂഡോമോണാസ് ഫ്ലൂറസെൻസ് (സീഡ് പ്രൈമിങ്- 10g/L ടാൽക് ഫോർമുലേഷൻ, ഫോളിയാർ സ്പ്രേ 30mL/L കൾച്ചർ ബ്രോത്ത്), എന്നിവ ഉപയോഗിച്ച എള്ള് ചെടികൾ മറ്റുള്ളവയെക്കാൾ കൂടുതൽ വെള്ളക്കെട്ടിനെ അതിജീവിക്കുകയും വളർച്ച കൂടുകയും ചെയ്തതായി കണ്ടു. മാത്രമല്ല ഇവ പ്രയോഗിച്ച ചെടികളിൽ ക്യാറ്റലേസ് എന്ന ആന്റിഓക്സിഡന്റും ഹരിതകവും അധികമായി കാണുകയും, മെലൺഡൈആൾഡിഹൈഡ് കുറയുന്നതായും കണ്ടെത്തുകയുണ്ടായി.

ചട്ടികളിൽ നടത്തിയ ഈ പരീക്ഷണത്തിൽ നിന്നും തിരഞ്ഞെടുക്കപ്പെട്ടവയെ 2019 ഫെബ്രുവരി-മെയ് മാസങ്ങളിലായി ആലപ്പുഴ ജില്ലയിലെ നിലങ്ങളിൽ പരീക്ഷിക്കുകയുണ്ടായി. പൊട്ടാസിയം നൈട്രേറ്റ് (5g/L), സാലിസിലിക് ആസിഡ് (100ppm),സ്യൂഡോമോണാസ് ഫ്ലൂറസെൻസ് (സീഡ് പ്രൈമിങ്- 10g/L ടാൽക് ഫോർമുലേഷൻ, ഫോളിയാർ സ്പ്രേ 30mL/L കൾച്ചർ ബ്രോത്ത്) എന്നിവ ഉപയോഗിച്ച് തിലക് എന്ന എള്ളിനത്തിൽ നടത്തിയ പരീക്ഷണത്തിൽ, 8 -17% വരെ വിളവർദ്ധനയുള്ളതായി കണ്ടെത്തി.

വരൾച്ചയെ അതിജീവിക്കാൻ കഴിവുള്ള എള്ളിനങ്ങളെ തിരഞ്ഞെടുക്കുന്നതിനായി ലാബിൽ PEG (പോളി എതിലീൻ ഗ്ലൈക്കോൾ) എന്ന രാസവസ്തു ഉപയോഗിച്ച് വിത്തുകളിൽ പരീക്ഷണം നടത്തി. വന്യ ഇനം എള്ള് (സെസാമം മലബാറിക്കം), TMV3, അയാളി, കായംകുളം1, തിലതാര എന്നിവ വരൾച്ചയെ ചെറുക്കാൻ താരതമ്യേന കഴിവുള്ളതായും C01, TMV5, TMV7 എന്നിവ വരൾച്ചയെ ചെറുക്കാൻ കഴിവ് കുറവുള്ള എള്ളിനങ്ങളാണെന്നും കണ്ടെത്തി .

Z ഡിസ്ട്രിബ്യൂഷൻ അനാലിസിസ് വഴി എള്ളിനങ്ങളെ 6 ഗ്രൂപ്പുകളായി തരംതിരിച്ചു.

1. വെള്ളക്കെട്ടിനെയും വരൾച്ചയെയും അതിജീവിക്കാൻ കഴിവുള്ളവ – സെസാമം മലബാറിക്കം.
2. വെള്ളക്കെട്ടിനെയും വരൾച്ചയെയും അതിജീവിക്കാൻ കഴിവില്ലാത്തവ –C01.
3. മിതമായ രീതിയിൽ വെള്ളക്കെട്ടിനെയും വരൾച്ചയെയും അതിജീവിക്കാൻ കഴിവുള്ളവ – അയാളി, തിലറാണി, കായകുളം1, SVPR1.
4. വെള്ളക്കെട്ടിനെ അതിജീവിക്കുകയും വരൾച്ചയെ അതിജീവിക്കാൻ കഴിവില്ലാത്തവയും– TMV5, TMV7.
5. വരൾച്ചയെ അതിജീവിക്കുകയും വെള്ളക്കെട്ടിനെ അതിജീവിക്കാൻ കഴിവില്ലാത്തവയും– തിലതാര.

എള്ളിനങ്ങളിൽ നടത്തിയ മോളിക്കുലർ പഠനങ്ങളിൽ നിന്നും തിലതാര എന്ന എള്ളിനത്തിൽ ഫോസ്ഫോ ഇനോൾ പൈറുവേറ്റ് കാർബോക്സിലേസ് എന്ന ജീനിലും, C01 എന്ന എള്ളിനത്തിൽ സൈലോ ഗ്ലുകാൻ എന്റ്റോടാൻസ് ഗ്ലൈക്കോസിലെസ് എന്ന ജീനിലും വ്യതിയാനം ഉള്ളതായി കണ്ടെത്തി.

മേൽപ്പറഞ്ഞ രീതിയിൽ നടത്തിയ പരീക്ഷണങ്ങളുടെ അടിസ്ഥാനത്തിൽ സെസാമം മലബാറിക്കം, അയാളി, തിലറാണി, കായംകുളം1 എന്നിവ വരൾച്ചയെയും വെള്ളക്കെട്ടിനെയും അതിജീവിക്കാൻ കഴിവുള്ളവയായതിനാൽ, ഈ ഇനങ്ങളെ ക്രോപ് ഇമ്പ്രൂവ്മെന്റ് പ്രോഗ്രാമിൽ ഉൾപ്പെടുത്താവുന്നതാണ്. സ്യൂഡോമോണാസ് ഫ്ലൂറസെൻസ്, പൊട്ടാസിയം നൈട്രേറ്റ്, സാലിസിലിക് ആസിഡ് എന്നിവ വെള്ളക്കെട്ടു മൂലം എള്ള് ചെടിയിൽ ഉണ്ടാകുന്ന പ്രശ്നങ്ങളെ അതിജീവിക്കാൻ ഉപയോഗിക്കാവുന്നതാണ്.