PHYSIOLOGICAL CHARACTERIZATION OF THERMO-SENSITIVE GENIC MALE STERILITY IN RICE (*Oryza sativa* L.).

by

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(2014-11-181)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Physiological characterization of thermo-sensitive genic male sterility in rice (*Oryza sativa* L.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Physiological characterization of thermo-sensitive genic male sterility in rice (*Oryza sativa* L.)"** is a record of research work done independently by MS. Neethu Chandra. C (2014-11-181) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associate ship to her.

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Neethu Chandra. C

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LIST OF ABBREVIATIONS AND SYMBOLS USED

RARS	Regional agricultural Research Station
IRRI	International Rice Research Institute
MS	Male sterility
WA	Wild abortive
CMS	Cytoplasmic male sterility
EGMS	Environment-sensitive genic male sterility
PGMS	Photosensitive genic male sterility
TGMS	Thermosensitive genic male sterility
PTGMS	Photothermosenitive genic male sterility
CSP	Critical sterility point
HSP	Heat shock protein
PCD	Programmed cell death
DNA	Deoxyribo nucleic acid
F_1	First filial
F_2	Second filial
F_3	Third filial
IKI	Iodopotassium iodide
IAA	Indole acetic acid
MDA	Malondialdehyde
SOD	Superoxidedismutase
DMSO	Dimethyl sulphoxide
PMS	Potassium metabisulphate
NBT	Nitroblue tetrazolium
NADH	N- adenosyl dihydrogen

FW	Fresh weight
TCA	Trichloroacetic acid
TBA	Thiobarbituric acid
pH	Hydrogen potential
RH	Relative humidity
ROS	Reactive oxygen species
CST	Critical sterility temperature
AAO	Ascorbic acid oxidase
IAAO	Indole acetic acid oxidase
Min	Minimum
Max	Maximum
min	Minutes
%	Per cent
cM	Centimorgan
µg	Microgram
^{o}C	Degree Celsius
m^{-2}	Per metre square
g^{-1}	Per gram
s^{-1}	Per second
cm	Centimeter
ml	Millilitre
M	Molar
ppm	Parts per million
µ	Micro
ha	Hectare

mg	Milligram
nm	Nanometer
mm	Millimeter
Fig.	Figure
g	Gram
rpm	Rotations per minute
et al.	and other Co workers
i.e.	That is
KAU	Kerala Agricultural University
A_{663}	Absorbance at 663nm
A_{645}	Absorbance at 645nm

Introduction

1. INTRODUCTION

Rice is the staple food for a large category of population all over the world. As reported by Gnanamanickam in 2009, rice is life, for most of the people living in Asia. Rice has shaped the cultures, diets and economies of thousands of millions of people. Considering its important position, the United Nations designated year 2004 as the International Year of Rice. Devoting a year to a commodity was unprecedented in United Nations history. However, the 57th session of the United Nations General Assembly noted that rice is the staple food of more than half of the world's population, affirmed the need to heighten the awareness of the role of rice in alleviating poverty and malnutrition and reaffirmed the need to focus world attention on the role, rice can play in providing food security and eradicating poverty. In India, green revolution contributed towards the production of food grains including rice. According to recent data, in India 43 million hectare area is under rice cultivation, with about 105 million tons production (GOI, 2015). Yield has almost reached a plateau for most of the high yielding varieties. As the population is increasing in a drastic scale, this yield plateauing will adversely affect the food security of our nation until and unless we adopt some newer technologies that can improve and can assure food security in the future.

Kerala is one of the southern states of our nation. In Kerala, people mainly consume rice for all the three meals of a day. Our state is having a total area of 1, 99,611 ha under rice in 2013 -14 (GOK, 2015) and is having a production of 564325 tons. There is a 20 % decrease in production when compared with the production in 2001-2002 period. We mainly depend on our neighbouring states especially Andhra Pradesh and Tamil Nadu for rice so as to meet the demand supply gap. Another speciality with rice consumption in Kerala is that, Keralites mainly prefer red rice (rice with red to brown bran) over white, which are bold and nonsticky in nature. Red rice is also having higher nutritional quality than that of white rice.

One of the best options to improve rice production is to exploit hybrid vigour as proved in China. As rice is a self pollinated crop, the possible way of hybrid production is to go for male sterility system. In India, cytoplasmic male sterility system is already in use for hybridization programme. Totally 46 hybrids have been released for commercial cultivation in the country. Among these, 29 have been released from the public sector while remaining 17 have been developed and released by the private sector (Viraktamath *et al*., 2011). As the CMS character is controlled by mitochondrial factors, a wild abortive (WA) type of cytoplasm is necessary. This is difficult to be achieved, due to the absence of WA cytoplasms according to the requirement, especially in red rice background. Other system includes environment-sensitive genic male sterility (EGMS) which includes PGMS and TGMS. EGMS is a male sterility system where the male sterile gene expression is influenced by environmental factors such as temperature (TGMS) or day length (PGMS), or both (PTGMS). Thermosensitive genic male sterility system is a better option over CMS as it can be transferred to the progeny by crossing, since the trait is controlled by nuclear genes. The maintenance of male sterile line in TGMS system is relatively easier than in CMS system as it doesn't need a maintainer line. The TGMS plant will be male sterile as well as male fertile depending on the temperature conditions, i.e, fertile at low temperatures and sterile at high temperatures. With respect to Kerala conditions, TGMS system is more adoptable as we are having significant temperature variation between seasons and also between altitudes. So the seed multiplication will also be possible in our state which makes the hybrid seed production more easy. While considering yield also, two line hybrids are having an yield advantage of 30 % where it is 15–20 % in three line breeding, over the best conventionally bred varieties.

For a tropical region like Kerala, TGMS is ideal and the pre-requisite is to develop male sterile line with stable sterility. Hence this study is an attempt to

develop, thermosensitive genic male sterile line in red rice background and to evaluate its physiological and phenological characters.

Review of literature

2. REVIEW OF LITERATURE

2.1 RICE

Rice is a monocot plant of the family Poaceae, domesticated by humans, years back. By the process of domestication only, there evolved large number of rice cultivars in almost all rice growing tracts with different conditions including climate, soil, water availability and topography (Takahashi, 1984). Oka (1988) reported that the propagation of wild rice is independent to humans while cultivated ones are more dependent to human intervention. It is one of the most important food crop consumed by more than half of the world population (Sasaki and Burr, 2000). The preference for rice varies among people. Most of the world population prefers white rice but in Kerala, the southern state of India, prefer red rice and generally bold grains. The red rice is superior in nutritional quality also, when compared to the white rice with higher calories, protein etc.

In India, area under rice is showing a declining trend, from 440.06 lakh ha in 2011-2012 period which went down to 439.49lakh ha in 2013-2014 period which further went down to about 435 lakh ha in 2015 (GOI, 2015). Regarding production, it stagnated around 45-47 million tons for last five years. After green revolution there was a hike in production of food crops, especially cereals. This trend has recently changed due to yield plateauing (GOI, 2015). A similar trend is visible while considering Kerala also; where, there was 38 % reduction in area from 2001-2014 and 20 % reduction in grain production (GOK, 2015). This stagnation in yield can lead to severe food scarcity as the alarming rate of population growth will lead to greater demand, and thereby an increase in the demand supply gap. Hereafter to meet this gap in demand and supply, we should be able to produce more. This can be achieved by utilizing the hybrid vigour or heterosis.

2.2 HYBRID RICE

Hybrids are nothing but the F_1 seeds produced by crossing two parents that are least related. In rice, heterosis was observed in 1926 (Jones, 1926; Ramiah, 1933). In 1966, Yuan Long-ping, father of hybrid rice in China, initiated the attempts for the adoption of hybrid technology (Yuan, 1997). Virmani *et al.* (1997) and Yuan in 1997 reported that hybrid rice is having 15 -20 % of higher grain yield. Virmani in 2003 also reported that hybrid vigour and the efficiency in the technique used for the generation of hybrid determine the extent of success in hybrid rice. As reported by Viraktamath *et al.* (2011), by the continuous effort of two decades, 46 hybrids were released for cultivation in India, out of which 29 were from public sector and 17 were developed and released from public sector. Some of them have been outdated, and some are not in the production chain.

2.3 MALE STERILITY

Male sterility can be defined as a condition in which the pollen grain is unviable or cannot germinate and fertilize normally to set seeds.

According to Govind and Virmani (1988) pollen grains which are unstained, withered or spherical are sterile and the lightly stained round pollen grains were also grouped under sterile.

Male sterility is basically classified into three, they are, cytoplasmic genetic male sterility (CMS), environment-sensitive genic male sterility (EGMS), chemically induced male sterility

In CMS, male sterility is controlled by the male sterility factor S in mitochondrial DNA. There will be three lines namely A line, B line and R line which are CMS line, its maintainer line (the female counter part) and the restorer line respectively.

EGMS is a male sterility system where the expression of male sterility is influenced by environmental factors such as temperature (TGMS) or day length

(PGMS), or both (PTGMS) and is regulated by nuclear genes. As this MS system is controlled by recessive nuclear genes they can easily be transferred (Virmani, 2003)

2.4 THERMOSENSITIVE GENIC MALE STERILITY

TGMS lines are those lines which alter their sterility or fertility expression according to temperature (Virmani, 2003).

Good knowledge about the fertility behaviour of TGMS will help to effectively utilize this novel male sterility system, since the nuclear sterile gene reacts differently to temperature based on genetic factors (Viraktamath and Virmani, 2001). Identification of TGMS lines with stable fertility-transformation behaviour is the first step required in the utilization of the two-line system of hybrid rice breeding on a large scale (Salgotra *et al*., 2012)

The lines showing 100 % pollen sterility at high temperature and self-seed set of more than 30 % at low temperature can be taken as TGMS lines for commercial purpose (Vijayalakshmi and Bangarusamy, 2007)

According to Maruyama *et al.* (1991), TGMS is the best alternative over CMS considering seed production. This is because TGMS is two-line system whereas CMS is three-line system (Wang *et al.,* 2003). TGMS line shows male sterility with respect to temperature change. Developmental stage at which the plant is subjected to temperature variation is critical for the expression of sterility or fertility in TGMS (Dong *et al*., 2000; Reddy *et al*., 2000)

Hybrid rice production by three-line breeding where CMS parental lines are used is very difficult as it limits the use of many parental lines due to the selective occurrence of restorer genes. In TGMS, two-line hybrid system, frequency and spread of heterotic hybrids are higher as we are free to use any male fertile line as a male parent and it is devoid of the negative effects of sterility inducing cytoplasm (Lopez and Virmani, 2000).

According to He *et al*. (1999), selection of lines with TGMS character is difficult under field or controlled conditions as this TGMS trait is known to be influenced by (*Fd*) fertility differential genes, and epistatic interactions.

Accessibility to TGMS lines with a superior genetic background and appreciably stable sterility and also good outcrossing potential will improvise two-line hybrid breeding programs. Seed yield is less with respect to hybrid rice technology which is one of its undesirability. By improving the natural outcrossing, the hybrid seed yield can be enhanced. Extent of outcrossing depends many floral characters such as panicle exertion, stigma exertion, duration of glumes opening, etc (Robin *et al.*, 2010)

2.5 THERMOSENSITIVE GENIC MALE STERILITY GENES

Several PGMS and TGMS genes have been identified to be present on various chromosomes of rice.

Four PGMS genes such as, *pms1*, *pms2*, *pms3* and *p/tms12-1*, are located on chromosome number; 7, 3, 12 and 12, respectively (Zhang *et al*., 1994(a); Mei *et al*., 1999; Lu *et al*., 2005; Zhou *et al*., 2012).

Three reverse PGMS genes, *rpms1*, *rpms2* and *CSA*, have been mapped to chromosomes 8, 9 and 1, respectively (Peng *et al*., 2008; Zhang *et al*., 2013). Nine TGMS genes, *tms1* (Wang *et al*., 1995), *tms2* (Yamaguchi *et al*., 1997; Lopez *et al*., 2003; Pitnjam *et al*., 2008), *tms3* (Subudhi *et al*., 1997), *tms4* (Dong *et al*., 2000), *tms5* (Wang *et al*., 2003; Yang *et al*., 2007 a, b), *tms6* (Lee *et al*., 2005), *TGMS* gene from SA2 (Reddy *et al*., 2000), *ptgms2-1* (Xu *et al.,* 2011) and *tms9* (Sheng *et al*., 2013), have been mapped to chromosomes 8, 7, 6, 2, 2, 5, 9, 2 and 2, respectively.

One reverse TGMSgene, *rtms1* from J207S, has been mapped to chromosome10 (Jia *et al*., 2001)

According to the findings of Qi *et al*. (2014), from the study done on Hengnong S-1, a male sterile rice widely used in China (Zhou *et al*, 1988), the *tms9-1* gene was restricted to the region between QY-9-19 and QY-9- 27, and the genetic

distance was 0.22 and 0.07 cM, respectively. The *tms9-1* gene was finally mapped on a 162.1 kb region in the BAC clone AP005308 and AP005575. According to the annotation database of this region, there was a putative *MALE STERILITY1* (*OsMS1*) in this region

2.6 **OUT CROSSING**

Rice is a self pollinated crop which can show about 1 % of out crossing (Beachell *et al*., 1938).

The propagation of TGMS lines and seed generation of two-line hybrids is easier than that of three-line hybrids, as in the later, a maintainer line is required in order to multiply the CMS line whereas TGMS lines become fertile under low temperature conditions and thus self fertilize to produce seeds.

The sterile- and fertile-sensitive phases of TGMS lines need to be determined for different ecological areas, so that the proper locations for sterile line multiplication and hybrid seed production can be established (Salgotra *et al*., 2012).

According to Virmani (2003), in hybrid rice seed production, outcrossing potential of parental line is one of the factors that determine the seed yield. For the male sterile parental line outcrossing is reported as a function of floral morphology as well as flowering behaviour (Oka and Morishima, 1967). In order to improve the seed yield, proper, timely and sufficient deposition of pollen on to the receptive stigma of the female spikelet, which is male sterile. Karpagam (2011) reported that, outcrossing ability of TGMS is proportional to the stigma length. In 1993, Ali reported that, the maximum anther length and stigma length in TGMS is having a positive correlation with outcrossing rate. According to Virmani (2003) fertility chances under favourable conditions is less in lines having larger pollen grains. To produce potential hybrids, the development of parents with a longer duration is essential (Robin *et al*., 2010)

High outcrossing rate is the most important factor which is considered while adaptability of hybrid seed production is evaluated (Abeysekera *et al*., 2003)

2.7 EVALUATION FOR STABLE STERILITY

Selecting TGMS lines with very low CSP (critical sterility point) is suitable. It will prevent reversion of sterility in TGMS by sudden temperature fluctuations. This will help to avoid contamination of TGMS selfed seeds in hybrid seed lot in a hybrid population and thereby ensures purity. According to Celine (2015) the CSP is the critical temperature during the sensitive stage of a TGMS line that results in complete sterility. Ying (1999), reported that for TGMS lines, mean daily temperature is the best CSP. According to Viraktamath and Virmani (2001), the sterility and fertility condition of TGMS grown in tropical region is influenced by maximum temperature.

Ali *et al*. (1995) reported that 15-24 days prior to heading is the sensitive stage of rice which merges with the period of development of rice PMC (pollen mother cell) to the formation of secondary branch primordia. Ali *et al*. (1995) also mentioned that different TGMS lines may have different CSP.

2.8 TEMPERATURE SENSING BY PLANTS

Temperature is a very important factor that controls plant growth, metabolism and development. As plants are sessile organisms, it necessitates their need to recognize any change in their ambient temperature. Physiological and biochemical findings suggest the existence of specific calcium-permeable channels that respond to heat or membrane fluidizers. Within minutes of a temperature rise, a conserved transient calcium influx is observed in several plants (Gong *et al*., 1998; Liu *et al*., 2006; Saidi *et al*., 2009; Wu and Jinn, 2010). Ca^{2+} involved in heat signalling is extracellular (Saidi *et al*., 2009).

Plant thermosensors are heat-responsive Ca^{2+} channels in the plasma membrane (Saidi *et al*., 2009). These calcium channels can be classified into two,

they are- voltage dependent calcium channels and voltage independent calcium channels. Voltage dependent calcium channels include hyper polarisation activated calcium channels (HACC) and depolarisation activated calcium channel (DACC). Voltage independent calcium channels include voltage independent calcium channels (VICC) and Mechanosensitive calcium channels (MSCC) (Miedema *et al*., 2001; Chen *et al*., 2012). HACC, VICC & DACC are present on the plasma membrane. MSCC are present on the leaf, root, and pollen plasma membrane. There are endo-membrane calcium channels on the membrane of vacuole, endoplasmic reticulum, chloroplast and nucleus. At the vacuole, TWO PORE CHANNEL1 (TPC1) is the slow vacuolar (SV) Ca^{2+} release channel in *Arabidopsis* (Peiter *et al*., 2005). Glutamate receptor (GLR) and cyclic nucleotide gated calcium channels (CNGC) gene families which are the most likely sources of genes encoding HACCs, DACCs, and VICCs (Dietrich *et al*., 2010; Dodd *et al*., 2010; Jammes *et al*., 2011; Hedrich, 2012).

In signalling various components such as calmodulin, calmodulin binding kinase, hydrogen peroxide, nitrous oxide, HSP 90, heat shock transcription factors, heat shock proteins etc are involved (Saidi *et al*., 2010).

2.9 BIOCHEMICAL CHANGES

According to Stewart *et al.* (1980), better understanding of protein will help to understand the metabolism as they are gene products. So by studying the protein distribution in both sterile and fertile plants will help us understand the difference in their metabolism. Changes can occur in amino acids content, nitrogen compounds etc with change in environmental conditions. Peng and Wang (1991) reported that sugar accumulation in plants during stress will help to mitigate the stress damage by providing osmotic effect. Similarly Peng and Wang (1991) reported the accumulation of proline with stress and accumulation was reported to exhibit a positive relation with the duration of stress exposure. During adverse stress conditions, total phenols are normally produced by plants as metabolic byproducts and according to Rodionova *et al*. (1995), phenolic compounds and high temperature condition were associated.

By understanding the biochemical changes in TGMS plants during sterility transformation influenced by environmental conditions will be helpful for utilizing the system in a desirable way stress (Vijayalakshmi and Bangarusamy, 2007).

Reduced amino acid availability as well as low nitrogen assimilation could lead to male sterility in lines as the former can cause low soluble protein content (Peng and Wang, 1991; Huang, 1994; Stephen and Thangaraj, 2000).

According to Khan (1973), male sterile plants accumulated both proline and hydroxyproline. Luo (1988) put forth the idea of using proline content as an index for male sterility. According to Vijayalakshmi and Bangarusamy (2007), in critical sterility temperature treated plants of TS 29 there was a steep decrease in total free amino acid contents which they suggest as a possible reason for causing pollen sterility. In 1998, Stephen reported a reduction in amino acid pool in TS 16 and TS 18 when subjected to critical sterility temperature treatment.

2.10 MECHANISM OF TGMS

Male reproductive development in plants involves several major developmental stages in series and along several cell lineage pathways, which include specification of stamen primordia, production of sporogenous cells, development of tapetum and microspore mother cells (MMCs), meiosis, formation of free haploid microspores, degeneration of tapetum and release of mature pollen grains (Goldberg *et al*., 1993). If any of the above mentioned steps are adversely affected, then it can possibly lead to male sterility (MS), the failure to produce or release functional pollen grains.

The phenotypic appearance of male sterility can include complete absence of male organs, abnormal sporogenous tissues, inability of anther to dehisce or even the inability of pollen to germinate on compatible stigma (Chase *et al*., 2010).

The correct timing of tapetal PCD is important, and premature or delayed PCD is often associated with male sterility. Most of the rice male sterile mutants, exhibit delayed tapetal PCD (Li *et al*., 2006; Ji *et al*., 2013). Some EGMS and WA-CMS rice have premature tapetal PCD (Ding *et al*., 2012; Luo *et al*., 2013;) Premature tapetal PCD starts as early as the microspore mother cell (MMC) stage (stage 6) and it continues until the tapetal cells are completely degraded in Annong S-1, grown under high temperature conditions (Ku *et al*., 2003). Mutations in such genes often result in male sterility in different forms, e.g. knockout mutation of CAP1, which encodes L-arabinokinase, resulted in collapsed abnormal pollens (Ueda *et al*., 2013). Microsporeless anthers resulted from null mutations of MSCA1 in corn (Chaubal *et al*., 2003) and MIL1 in rice (Hong *et al*., 2012). A nonsense mutation of OsaTRZ1 (RNZm) could be responsible for the TGMS traits in rice (Zhang and Yang, 2014). Cytological study of the development of the Zhu1S anther wall and microspores showed that tms9 encoded male sterility is due to the starving of microspores and thereby leading to pollen abortion where the tapetum failed to degenerate normally,(Sheng *et al*., 2015). According to Guo and Liu (2012) and Wang *et al*., (2013), more than 40 male sterile genes have been cloned in rice

Chen *et al*., (2007) has proposed that, in some case, change of TGMS from sterility to fertility may be due to temperature-sensitive RNA splicing. They also reported two homologous *UGPase* genes (*Ugp1* and *Ugp2*) which are present in rice genome. *Ugp1* silencing by RNA interference or co-suppression resulted in male sterility. More interestingly, *Ugp1*-co-suppressing plants contained unprocessed introns containing primary transcripts derived from transcription of the over expression construct, and these aberrant transcripts undergo temperature sensitive splicing in florets, leading to a novel thermo-sensitive male sterility.

Materials and methods

3. MATERIALS AND METHODS

The experiment entitled "Physiological characterization of thermo-sensitive genic male sterility in rice (*Oryza sativa* L.)" was undertaken with an objective to develop TGMS rice in red rice background and to evaluate the physiological and phonological characterization. To achieve these objective plants were raised in pots at Department of Plant Physiology, College of Agriculture, Vellayani (2014-2016). Phenological observations were made during flowering and biochemical observations were made at the time of flowering stage in sterile and fertile plants.

3.1 EXPERIMENT DETAILS

3.1.1 Location of work

College of Agriculture, Vellayani situated at 8°5'N latitude and $76^0$9'E longitude and an altitude of 29 m above mean sea level. Seed multiplication was done at Regional Agricultural Research Station, Ambalavayal, Wayanad which is at $11^0$6' N latitude and $76^0$2' E longitude and 974 m above mean sea level.

3.1.2 Planting material

Seeds of rice varieties Uma, Jyothi and a TGMS line from IRRI (EC 720903), were used as parents for crossing. F_1 and F_2 generations from the parental crosses were also used.

3.1.3 Experimental methodology

3.1.3.1 Development and evaluation of male sterile F_2 plants

The available TGMS line (EC 720903) and suitable red rice varieties (Uma, Jyothi) were grown in pots (sowing started on 10-11-2014. *i.e*, 45th standard week of 2014) and crossed using proximal method (keeping male and female parents side by side on the day of flowering and covering the panicles together) to obtain F_1 progeny

which was selfed to get F_2 and then evaluated for male sterility. Plants were maintained with proper irrigation, fertilization and pest and disease management. Date of first flowering, date of 50 % flowering, date of crossing, date of maturity and pollen sterility were noted. Pollen sterility was analysed using 1 % IKI solution, which was prepared using 2.5 g KI and 250 mg iodine made up to 125 ml. Anthers were collected from flowers on or just before anthesis, on to which 2-3 drops of 1 % IKI was added after keeping on a glass slide. Stained pollen are fertile where unstained are sterile. Spikelet sterility was also evaluated.

3.1.3.2 Physiological and phenological characterization of thermo-sensitive male sterile line

The TGMS line (F_2) developed were used for this experiment. Plants were sown at monthly interval to identify the critical sterility temperature, which is taken as the mean temperature 15 to 30 days prior to heading. They were evaluated for their physiology and phenology. As and when a plant was found to be male sterile, it was taken to RARS Ambalavayal so as to confirm TGMS character and also to obtain seeds from ratoon. Collected seeds were observed for red colour also. The season suitable for hybridization or seed multiplication were also identified by comparing with the weather data of Vellayani as well as RARS Ambalavayal. Pollen sterility and spikelet sterility of these plants were monitored. Physiological and phonological characterizations were also done.

Date of first flowering, date of 50 % flowering, pollen sterility, spikelet sterility, total free amino acids and weather parameters such as temperature, humidity and rainfall were observed.

3.1.3.3 Analysis of physiological mechanism of male sterility

The TGMS plants (EC 720903) were maintained at sterility inducing conditions and fertility inducing conditions simultaneously. Samples were collected

from both the systems at flowering stage to analyse the physiological and biochemical parameters such as, chlorophyll content, amino acids, phenolic compounds, superoxide dismutase (SOD), ascorbate content, malondialdehyde (MDA), bioassay of IAA and total soluble proteins.

3.2 OBSERVATIONS

3.2.1 Physiological and phenological observations

3.2.1.1 Date of first flowering

Date on which the plants sown on the same day flowered for the first time was noted as the date of first flowering.

3.2.1.2 Date of 50 % flowering

Date on which 50 % of the plants flowered was noted.

3.2.1.3 Date of crossing

The date on which parent plants were crossed was noted.

3.2.1.4 Date of maturity

Date of maturity is the date of seed collected from the plants

3.2.1.5 Pollen sterility

Pollen sterility was evaluated using 1 % IKI solution. The plants were evaluated during their flowering period and anthers were collected from the plants during flowering, stained by keeping the anthers in the solution on glass slide for 1-2 minutes and were observed under the *Leica* microscope

3.2.1.6 Spikelet sterility

It was evaluated by covering the panicle during flowering and then after seed set, the panicles were collected and counted for set seeds and unset seeds. This was expressed in terms of unset seeds to total as spikelet sterility.

3.2.1.7 Weather parameters

Weather data from 2014 to 2016 June was collected from the Department of Meteorology, College of Agriculture Vellayani and 15 year weather data from RARS Ambalavayal was also collected.

3.2.1.7.1 Temperature

Mean of maximum temperature and minimum temperature were calculated separately for standard weeks and the mean of maximum and minimum temperature was also calculated for standard weeks for the duration of 2014 to 2016 with respect to Vellayani condition. Average of fifteen year temperature was noted on monthly basis. Temperature was noted in ^{0}C.

3.2.1.7.2 Humidity

Maximum and minimum humidity were represented as %. It was calculated for average on standard week basis for Vellayani. Ambalavayal data was shown as average of fifteen years on monthly basis.

3.2.1.7.3 Rainfall

Average rainfall for standard weeks was represented in mm with respect to Vellayani condition. Rainfall at Ambalavayal was shown as fifteen year average on monthly basis in mm.

3.2.2 Biochemical observations

3.2.2.1 Estimation of chlorophyll (DMSO method)

0.1 g of weighed fresh sample was taken and cut into small bits and kept overnight in 10 ml of acetone (80 %): DMSO mixture (1:1 v/v) and the coloured solution was used for reading in spectrophotometer. Absorbance was read at 663 nm and 645 nm. The chlorophyll content was calculated as mgg^{-1} by substituting in the formula below.

$$\text{Chlorophyll a} = \left(12.7 \times A_{663} - 2.69 \times A_{645}\right) \times \frac{V}{1000} \times \frac{1}{fresh\,weight}$$

$$\text{Chlorophyll b} = (22.9 \times A_{645} - 4.68 \times A_{663}) \times \frac{V}{1000} \times \frac{1}{fresh\,weight}$$

$$\text{Total chlorophyll (a+b)} = (8.02 \times A_{663} - 20.2 \times A_{645}) \times \frac{V}{1000} \times \frac{1}{fresh\,weight}$$

3.2.2.2. Estimation of SOD

It was done as per the method described by Kakkar *et al*. (1984). Sample of 0.5 g was taken and ground with 3ml of potassium phosphate buffer, centrifuged at 2000 rpm for 10 min and the supernatants were used for the assay. The assay mixture contained 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of PMS, 0.3 ml of NBT, 0.2 ml of the enzyme preparation and water in a total volume of 2.8ml. Reaction was initiated by the addition of 0.2ml of NADH. The mixture was incubated at 30^0C for 90 seconds and arrested by the addition of 1ml of glacial acetic acid. This mixture was then shaken with 4ml of n-butanol, allowed to stand for 10 minutes and centrifuged. Intensity of chromogen in the butanol layer was measured in spectrophotometer at 560 nm. One unit of enzyme activity is defined as the amount of enzyme that gave 50 % inhibition of NBT reduction in1 minute.

3.2.2.3. Estimation of ascorbic acid

It was done volumetrically as explained by Harris and Ray (1935). 5 ml of 100 ppm working standard was pipette out into 100ml conical flask. 4% oxalic acid was added to it and was titrated against the dye (V_1 ml). Appearance of pink colour was noted as the end point. 0.5 to 5 g sample was taken and ground using 15 ml of 4 % oxalic acid. The homogenate was filtered and made up to a known volume and then centrifuged at 10,000 rpm for 10 min. the supernatant was made up to 25 ml using oxalic acid. 10 ml of 4 % oxalic acid was added to 5 ml of pipetted aliquot. This was titrated against dichlorophenol indophenols (DCPIP) solution till the end point (V_2 ml). The amount of ascorbic acid is calculated using the formula:

$$\text{Ascorbic acid} = \frac{0.5mg}{V_1 ml} \times \frac{V_2}{5ml} \times \frac{100}{weight\ of\ sample}$$

3.2.2.4. Estimation of phenol

Folin- Ciocalteateau method by Mayr *et al.* (1995) was used for the estimation of phenol. 0.5 g of the sample was ground with 10-times volume of 80 % ethanol. The homogenate was centrifuged at 10000 rpm for 20 min. The residue was re-extracted with 5 times the volume of 80 % ethanol, centrifuged and the supernatant was pooled. The supernatant was evaporated to dryness, and then residue was dissolved in 5 ml distilled water. 0.2 ml was pipetted out from this into a test tube and the volume was made upto 3 ml using distilled water to which 0.5 ml of folin-ciocalteateau reagent was added. After 3 min, 2 ml of 20 % of Na_2CO_3 solution was added. After mixing well, it was kept in boiling water bath for 1 min. After cooling to room temperature, absorbance was read at 650 nm against a reagent blank. From the standard curve of catechol, the concentration of phenol was calculated as mgg^{-100}.

3.2.2.5 Estimation of proline

Acid ninhydrin method by Bates *et al*. (1973) was done for the estimation of proline. 0.5 g fresh leaves were homogenized in 10 ml of 3 % aqueous sulphosalicylic acid, and then centrifuged at 6000 rpm for 15 minutes. 2 ml aliquot of supernatant was mixed with an equal amount of acetic acid and ninhydrin and heated in boiling water bath for 1hour. The reaction was terminated on ice bath and extracted with 4ml of toluene. The extract was vortexed for 20 seconds and the chrometophore containing toluene was used for reading absorbance at 520 nm with toluene as blank. A standard curve was drawn using concentration versus asorbance. The concentration of proline was determined from the graph and expressed as

µ moles per g tissue = {[(µg proline/ml) x ml toluene] / 115.5} x(5/ g sample)

where 115.5 is the molecular weight of proline.

3.2.2.6 Estimation of total free amino acids

Ninhydrin method (Moore and Stein, 1948) was followed for the estimation of total free amino acids. 0.5g of the plant sample was ground and homogenized with 5-10 ml of 80 % ethanol. The solution was centrifuged. The supernatant was then collected and the volume was reduced by evaporation and was used for the estimation of total free amino acids. 1ml of ninhydrin solution was added to 1ml extract and the volume was made up to 2 ml with distilled water. The test tubes were heated in boiling water bath for 20 min. The contents were mixed well after the addition of 5 ml diluents. Intensity of purple colour was read at 570 nm in spectrophotometer against a blank. Blank was prepared using 0.1 ml of 80 % ethanol, instead of extract. The concentration of total free amino acids in the sample was determined from a standard curve of leucine and was expressed as % equivalent of leucine.

3.2.2.7 Estimation of total soluble proteins

Total soluble proteins were estimated using Bradford method (1976) with bovine serum albumin as the standard. A series of protein samples were prepared in PBS. The experimental samples were prepared in 10 micro liters of PBS. A known volume of diluted dye binding solution was added to each test tube. The solution was mixed well and allowed to develop a blue colour for atleast 5 min but no longer than 30 min. The red dye turns blue when it binds protein and its absorbance was measured at 596 nm. Standard curve was constructed using absorbance versus concentration. Protein in the sample was calculated from the curve and expressed as mgg^{-1} FW.

3.2.2.8 Estimation of MDA

Lipid peroxidation was estimated using TBA reaction described by Bishayee and Balasubramaniam (1971). 100 micro litres of tissue homogenate in tris buffer of pH 7 was incubated in a reaction mixture containing KCl (100 micro L), ascorbic acid, ferrous ammonium sulphate and tris buffer for 1hr at 37^0C. After incubation 1ml of TCA was added and mixed thoroughly. Heated with 2ml of TBA in boiling water bath for 15 min. Mixture was allowed to cool and then centrifuged at 2000 rpm. The supernatant was read for absorbance at 530 nm. The amount of malondialdehyde formed was calculated from a standard curve of malondialdehyde.

3.2.2.9 Bioassay of IAA

Wheat coleoptiles elongation bioassay of IAA was done with wheat coleoptiles. Coleoptiles of germinated wheat were taken and incubated in plant extract prepared as follows. 0.1 g sample was taken and then extracted using 80 % ethanol and the supernatant was collected after centrifugation. 1ml of the extract was diluted to 10 ml. To this the 0.5 cm long coleoptiles were incubated. Distilled water was taken as control. The quantity of IAA was calculated by plotting a standard curve of IAA.

3.2.2.10 Transpiration rate

Transpiration rate was measured using the SAI-1 Porometer of company Delta T Devices and expressed as mmoles $m^{-2}s^{-1}$. Reading was taken with the index leaf at flowering stage of the crop.

a b

Plate 1. Germinating seeds (a, b) of 03 and F_2 of 03 X Jyothi

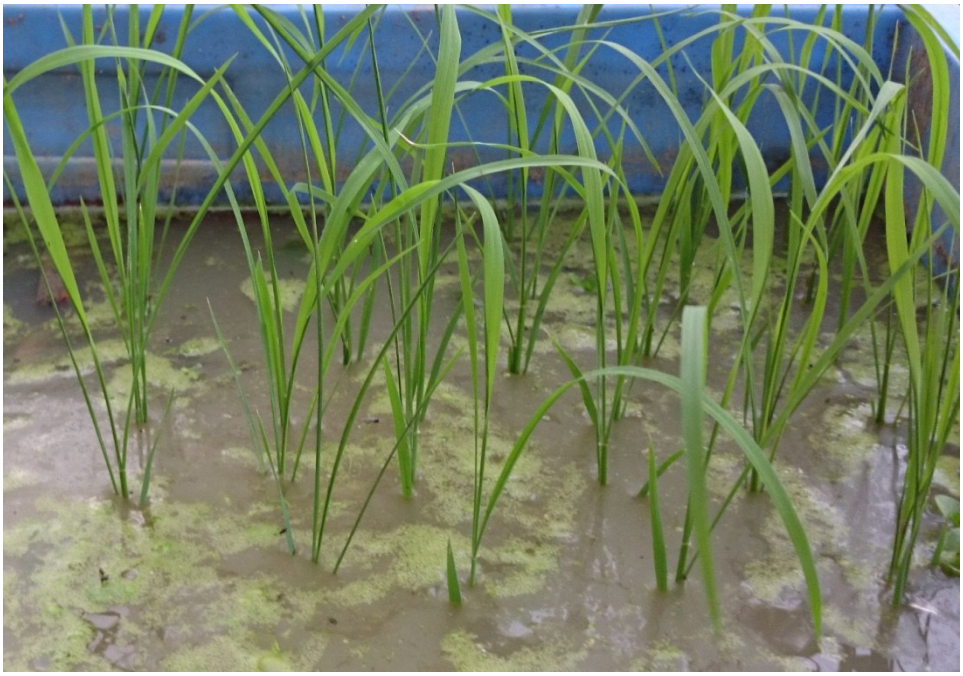

Plate 2. Transplanted seedlings of 03

Plate 3. Potted plants of F_2 plants of 03 x Jyothi

Plate 4. Flowering of 03

Plate 5. Crossing between 03 and Jyothi, Proximal method

Plate 6. Mature Seeds of 03 X Uma

Plate 7. Seed multiplication of TGMS 03 at RARS Ambalavayal

Results

4. RESULTS

As rice cultivation is under decline and requirement is increasing, developing a TGMS hybrid suitable for Kerala, i.e., in red rice background will be really helpful. Hence efforts are made in this study entitled "Physiological characterization of thermo-sensitive genic male sterility in rice (*Oryza sativa* L.)" to develop red TGMS seeds and also to evaluate the phenological characters of the TGMS lines. The results of various experiments conducted to achieve these objectives are presented in this chapter.

4.1 PHENOLOGY

4.1.1Phenology of parents

The dates of sowing, flowering (first flowering and 50 % flowering), crossing and maturity are presented in Table 1. The male fertile parent, Jyothi was showing a relatively earlier flowering than Uma and TGMS 03. The duration of TGMS plants were found to be around 120 days which is similar to the duration of male parents Uma and Jyothi. They exhibited an average of 5 days from first flowering to 50 % flowering. 50 % flowering in the male parent Jyothi was about 2 days earlier to that of Uma. Three to five crossings were possible in each plant and each lot, that too with either of the male parents. This was possible due to the synchronous flowering. Date of maturity is the date of seed harvested.

4.1.2 Pollen and spikelet sterility

Pollen and spikelet sterility of parental lines are presented in Table 2. The TGMS parent was found to be male sterile with 100 % spikelet sterility and pollen free anthers. With respect to the other parents, Uma exhibited 100 % pollen fertility in a few months where as the maximum pollen sterility observed was 3.3 % in 2015 February. The spikelet sterility of Uma was maximum in March 2015 with 34 % spikelet sterility and minimum of 22.2 % of spikelet sterility in February 2015. Jyothi exhibited a minimum pollen sterility of 0 % during April, June and July of 2015 and maximum of 2 % during December

2015. The minimum and maximum spikelet sterility was 21 % and 33.7 % respectively.

4.1.3 Phenology of F_2 plants.

The date of sowing, first and 50 % flowering are shown in Table 3. The F_2 plants showed a similar flowering behaviour as that of the TGMS parent. The number of sterile and fertile plants varied in different months as the seeds are from a bulk lot.

4.1.4 Pollen and spikelet sterility characters of F_2 plants.

Pollen and spikelet sterility characters of F_2 plants are shown in Table 4. The sterile F_2 plants were similar in their sterility character as their male sterile parent, i.e., the TGMS line, with 100 % spikelet sterility and pollen free anthers. The minimum pollen sterility exhibited by fertile F_2 plant was in those sown in the month of March 2015, i.e., 2.9 % and spikelet sterility in those sown in November 2014, i.e., 22 %. The maximum pollen sterility was recorded in January 2015 sown plants with 5.3 % pollen sterility and spikelet sterility of 30 % in May 2015 sown plants.

4.2 TOTAL FREE AMINO ACID CONTENT OF F_2 PLANTS.

The total free amino acid content of sterile and fertile plants of each month was noted down and is shown in the Table 5. It was found to be higher in sterile plants than in fertile during all seasons and the highest in the plants which were sown during the month of February (44.67 mg g^{-1}) and the least in those sown in the month of December (14.30 mg g^{-1}). Among fertile plants, the highest total free amino acid content was observed during July (7.06 mg g^{-1}) and lowest in December (6. 38 mg g^{-1}).

4.3 RED F_3 SEEDS FROM TGMS F_2.

The F_2 plants which were found to be sterile at Vellayani condition were taken to high altitude zone at RARS Ambalavayal which on seed set were

observed to be red in colour. 291 such F_3 seeds were obtained. On planting these seeds, F_3 plants were also found to be male sterile at Vellayani condition with pollen free anthers which is the character of the female parent EC720903 for selection. This is the major outcome of the present study.

4.4 WEATHER DATA AND CRITICAL STERILITY POINT

The weather data at Vellayani during the course of work showed that, at the time of flowering, the mean temperature was always above 26.1^0 C (Table 6, 7, 8). As the TGMS plants exhibited 100 % sterility with pollen free anthers during this time period, this temperature can be considered as the critical sterility point as reported by Ali *et al.* (1995). Critical sterility point is the temperature above which the plant shows 100 % sterility. Here as the plants showed 100% sterility throughout the year, the mean temperature was considered and the minimum of mean temperature was taken as the critical sterility temperature. As the plant was showing fertility and seed set at RARS Ambalavayal, after collecting the weather data of Ambalavayal we were able to plot the month wise graph for 15 years which shows that the mean temperature at Ambalavayal was always less than 25 ^{0}C (Table 9.). According to this data, it is clear that, it is possible to undertake hybridisation at Vellayani whereas we can go for seed multiplication at RARS, Ambalavayal as the mean temperature there is always less than 25 ^{0}C all during the year.

4.5 BIOCHEMICAL OBSERVATIONS

4.5.1 Chlorophyll content

Chlorophyll, the green colouring pigment in plants, was recorded and is shown in the Table 10. Total chlorophyll content was found to be higher in the sterile plants (1.26 mg g^{-1}), even though the content of chlorophyll b was lesser in sterile (0.39 mg g^{-1}) when compared to fertile plants (0.72 mg g^{-1}). The fertile ones exhibited higher chlorophyll a content than chlorophyll b.

4.5.2 Proline

Proline is an important amino acid that plants mainly accumulate during stress situations. The proline content obtained from the samples are shown in the Table 11. Here sterile plants were found to accumulate more proline than the fertile ones which indicated that they are experiencing stress. A mean amount of 33.82 µg g^{-1} of proline was accumulated by sterile plants whereas it was 9.18 µg g^{-1} in fertile ones.

4.5.3 Total soluble proteins

The total soluble protein content of the samples is shown in Table 12. Sterile plants were having a higher mean protein (17.56 mg g^{-1}) than in fertile plants (6.51 mg g^{-1}).

4.5.4 Total free amino acids

The total free amino acid content of sterile and fertile plants is shown in Table 13. As observed in the F_2 population, the 03 plants under sterility and fertility inducing conditions are also found to exhibit a similar trend with higher average amount of total free amino acids in the sterile plants (5.27 mg g^{-1}) and a much lesser amount in fertile plants (3.65 mg g^{-1}).

4.5.5 Malondialdehyde

Malondialdehyde is produced in the cells during stress as a result of membrane lipid peroxidation. The content of MDA directly relates to the stress that plants are exposed to. As shown in the Table 14, it was found to be higher in sterile plants (2.16 µg g^{-1}) than in fertile (1.54 µg g^{-1}). 40 % increase in MDA was found in sterile when compared with fertile.

4.5.6 SOD

Superoxide dismutase is an important enzymatic antioxidant which helps in scavenging free radicals produced due to oxidative stress. SOD content of sterile and fertile TGMS plants are shown in the Table 15, where, sterile plants had a higher amount of mean SOD activity (0.58 activity $g^{-1}min^{-1}$) than the fertile plants (0.31 activity $g^{-1}min^{-1}$).

4.5.7 Ascorbic acid

Ascorbic acid or vitamin C is an important non enzymatic antioxidant. The ascorbate content in sterile and fertile plant samples are shown in Table 16. It was found to exhibit a higher average content in sterile plants (46.87 mg g^{-100}) than in fertile plants (25.63 mg g^{-100})

4.5.8 Phenolic compounds

The content of phenolic compounds is shown in Table 17. A higher amount of phenolic compounds were present in the sterile plants (36.79 µg g^{-1}) than in fertile (15.47 µg g^{-1}).

4.5.9 IAA

Auxin content in the plant samples are given in the Table 18. The IAA content in sterile and fertile samples were comparable. It was found to be 0.25 µg g^{-1} in sterile plants whereas it was 0.23 µg g^{-1}in fertile

4.5.10 Photosynthetic rate

Photosynthetic rate is the amount of CO_2 fixed per unit area per unit time and was recorded and presented in Table 19. The TGMS F_3 of Jyothi had the highest photosynthetic rate of 39.40 µmol CO_2 m^{-2} s^{-1} and the least photosynthetic rate was exhibited by F_2 of Uma that is 1.70 µmol CO_2 m^{-2} s^{-1}. Uma had a photosynthetic rate of 10.57 µmol CO_2 m^{-2} s^{-1}, Jyothi with 10.07 µmol CO_2

$m^{-2}s^{-1}$, TGMS 03 with 7.57 µmol CO_2 m^{-2} s^{-1}, F_1 of Uma with 2.20 µmol CO_2 m^{-2} s^{-1}, F_2 of Jyothi with 4.70 µmol CO_2 m^{-2} s^{-1}

Table 1. Phenological characterisation of Uma, Jyothi and TGMS 03

Date of sowing	**Uma**		**Jyothi**		**Tgms 03**		**Date of crossing**	**Date of maturity**
	Date of first flowering	Date of 50 % flowering	Date of first flowering	Date of 50 % flowering	Date of first flowering	Date of 50 % flowering		
10-11-2014	07-02-2015	12-02-2015	06-02-2015	10-02-2015	07-02-2015	12-02-2015	7,8,9,14	12-03-2015
15-12-2014	09-03-2015	14-03-2015	09-03-2015	13-03-2015	10-03-2015	14-03-2015	10,13,14,15	04-04-2015
05-01-2015	31-03-2015	05-04-2015	31-03-2015	02-04-2015	31-03-2015	05-04-2015	2,4,5,7	10-04-2015
25-01-2015	20-04-2015	26-04-2015	20-04-2015	24-04-2015	20-04-2015	26-04-2015	22,25,26,27	26-05-2015
24-02-2015	20-05-2015	27-05-2015	20-05-2015	24-05-2015	20-05-2015	25-05-2015	20,23,24,25	30-06-2015
12-03-2015	06-06-2015	12-06-2015	05-06-2015	10-06-2015	06-06-2015	12-06-2015	6,7,12,13	10-07-2015
24-03-2015	25-06-2015	02-07-2015	25-06-2015	02-07-2015	25-06-2015	02-07-2015	27,28,4	05-08-2015
17-04-2015	12-07-2015	20-07-2015	12-07-2015	18-07-2015	12-07-2015	20-07-2015	12,18,19,25	30-08-2015
24-04-2015	22-07-2015	28-07-2015	22-07-2015	27-07-2015	22-07-2015	28-07-2015	25,26	04-09-2015
04-05-2015	06-08-2015	12-08-2015	06-08-2015	10-08-2015	06-08-2015	12-08-2015	7,8,9,15	17-09-2015
25-05-2015	22-08-2015	31-08-2015	22-08-2015	27-08-2015	22-08-2015	31-08-2015	29	30-09-2015
22-06-2015	23-09-2015	30-09-2015	23-09-2015	28-09-2015	23-09-2015	30-09-2015	25,26,27	29-10-2015

13-07-2015	10-10-2015	18-09-2015	10-10-2015	17-09-2015	10-10-2015	17-10-2015	10,11,17,18	25-11-2015
30-07-2015	04-11-2015	13-11-2015	05-11-2015	10-11-2015	04-11-2015	12-11-2015	5,7,8,14	15-12-2015
12-12-2015	03-03-2016	10-03-2016	03-03-2016	08-03-2016	03-03-2016	08-03-2016	8,9,10	12-04-2016
22-12-2015	20-03-2016	26-03-2016	20-03-2016	25-03-2016	20-03-2016	26-03-2016	20,22,26,27	30-04-2016
12-01-2016	06-04-2016	13-04-2016	06-04-2016	10-04-2016	06-04-2016	10-04-2016	6,9,10	15-06-2016
22-01-2016	19-04-2016	25-04-2016	19-04-2016	23-04-2016	19-04-2016	24-04-2016	20,23	30-05-2016
03-02-2016	20-05-2015	26-05-2016	18-05-2015	22-06-2016	17-05-2016	26-05-2016	22,25, 26	21-06-2016

Table 2. Pollen sterility and spikelet sterility characters of Uma, Jyothi and TGMS 03

Month of sowing	**Uma**		**Jyothi**		**Tgms 03**	
	Pollen sterility	Spikelet sterility	Pollen sterility	Spikelet sterility	Pollen sterility	Spikelet sterility
November 2014	F (0.00 %)	24.70	F (1.38 %)	27.00	100 %	100 %
December 2014	F (1.73 %)	25.00	F (1.80 %)	28.00	100 %	100 %
January 2015	F (2.90 %)	30.00	F (1.46 %)	28.00	100 %	100 %
February 2015	F (3.30 %)	22.20	F (1.00 %)	30.00	100 %	100 %
March 2015	F (2.70 %)	34.00	F (2.30 %)	32.61	100 %	100 %
April 2015	F (1.20 %)	32.00	F (0.00 %)	29.00	100 %	100 %
May 2015	F (0.00 %)	25.00	F (0.80 %)	27.20	100 %	100 %
June 2015	F (0.98 %)	30.00	F (0.00 %)	21.00	100 %	100 %
July 2015	F (0.00 %)	28.90	F (0.00 %)	21.17	100 %	100 %
December 2015	F (1.90 %)	29.60	F (2.00 %)	32.33	100 %	100 %
January 2016	F (2.80 %)	32.92	F (1.50 %)	27.00	100 %	100 %
February 2016	F (2.30 %)	27.00	F (1.80 %)	33.70	100 %	100 %

Table 3. Phenological characterisation of F_2 plants

Date of sowing	Date of first flowering	Date of 50 % flowering
10-11-2014	08-02-2015	12-02-2015
15-12-2014	09-03-2015	12-03-2015
05-01-2015	01-04-2015	07-04-2015
25-01-2015	21-04-2015	25-04-2015
24-02-2015	20-05-2015	26-05-2015
12-03-2015	06-06-2015	13-06-2015
24-03-2015	26-06-2015	02-07-2015
17-04-2015	12-07-2015	18-07-2015
24-04-2015	23-07-2015	28-08-2015
04-05-2015	06-08-2015	12-08-2015
25-05-2015	23-08-2015	31-08-2015
12-06-2015	08-09-2015	13-09-2015
22-06-2015	25-09-2015	30-09-2015
13-07-2015	12-10-2015	18-10-2015
30-07-2015	04-11-2015	10-11-2015
12-12-2015	05-03-2016	08-03-2016
22-12-2015	22-03-2016	25-03-2016
12-01-2016	08-04-2016	13-04-2016
22-01-2016	20-04-2016	23-04-2016
03-02-2016	18-05-2016	23-05-2016
11-02-2016	13-05-2016	23-05-2016

Table 4. Pollen and spikelet sterility characters of F_2 plants.

Month of sowing	Pollen sterility		Spikelet sterility	
	Fertile plants	Sterile plants	Fertile plants	Sterile plants
November 2014	F (3.9 %)	0	22.00	0
December 2014	F (4.6 %)	0	28.00	0
January 2015	F (5.3 %)	0	27.58	0
February 2015	F (4.5 %)	100 %	28.28	100 %
March 2015	F (2.9 %)	100 %	16.32	100 %
April 2015	F (4.0 %)	100 %	23.00	100 %
May 2015	F (4.0 %)	100 %	30.00	100 %
June 2015	F (3.2 %)	100 %	31.81	100 %
July 2015	F (3.7 %)	100 %	25.5	100 %
December 2015	F (4.0 %)	100 %	27.58	100 %
January 2016	F (4.4 %)	100 %	27.83	100 %
February 2016	F (5.0 %)	0	28.00	0

Table 5. Total free amino acid content of F_2 plants in mg g^{-1}

Month	Sterile	Fertile
February 2015	44.67	6.80
March 2015	28.44	6.39
April 2015	19.44	7.02
May 2015	20.98	7.00
June 2015	17.10	6.87
July 2015	19.60	7.06
December 2015	14.30	6.38
January 2016	37.20	6.87

Table 6. weather data of 2014 at Vellayani

Standard weeks	Temperature ^{0}C			Rainfall (mm)	RH %	
	Max.	Min.	Mean		Max	Min
1	30.9	21.5	26.2	0.0	94.9	77.6
2	29.0	22.3	25.6	14.0	94.4	77.4
3	31.0	21.8	26.4	0.0	94.1	76.1
4	31.3	20.7	26.0	0.5	90.4	69.9
5	31.4	21.9	26.7	0.0	92.3	68.6
6	30.7	20.2	25.5	0.0	95.1	68.9
7	31.4	22.8	27.1	3.0	92.0	72.0
8	31.5	23.8	27.6	9.0	90.6	70.6
9	31.9	23.1	27.5	12.5	92.3	68.6
10	31.9	23.4	27.7	0.0	90.4	66.9
11	32.4	21.4	26.9	0.0	93.0	63.4
12	33.0	24.1	28.5	3.3	93.7	69.1
13	33.0	22.2	27.6	0.0	89.1	64.0
14	32.4	24.5	28.5	9.5	89.9	75.3
15	32.0	24.2	28.1	16.0	91.0	69.6
16	32.0	25.0	28.5	6.0	90.7	73.3
17	32.8	24.4	28.6	11.4	94.0	76.6
18	32.2	23.8	28.0	18.7	93.1	83.3
19	30.7	24.3	27.5	33.1	92.0	84.4
20	32.5	25.1	28.8	0.0	88.3	72.6
21	32.4	25.5	28.9	8.3	86.3	75.6
22	31.8	25.6	28.7	1.8	92.5	75.2
23	30.1	24.5	27.3	4.9	94.3	83.3
24	30.7	25.1	27.9	3.3	90.9	77.4
25	31.1	25.7	28.4	2.8	92.3	77.7

26	30.5	25.0	27.8	4.8	92.7	79.1
27	30.4	24.7	27.6	2.5	90.9	79.0
28	29.7	24.2	27.0	4.8	92.9	80.4
29	30.1	24.2	27.1	6.5	90.4	76.7
30	29.9	24.2	27.1	3.7	91.6	73.6
31	29.2	23.5	26.3	15.7	95.3	85.9
32	29.4	23.5	26.4	22.2	88.6	77.3
33	29.7	24.0	26.9	2.0	89.7	79.6
34	29.8	24.0	26.9	73.0	94.0	80.9
35	29.9	23.9	26.9	34.4	87.6	84.1
36	29.2	23.9	26.5	16.0	96.1	79.3
37	30.1	24.5	27.3	1.5	89.3	74.1
38	30.5	24.6	27.6	0	85.0	75.6
39	31.1	24.1	27.6	18.6	93.3	84.9
40	30.7	23.9	27.3	3.0	95.4	73.6
41	30.7	24.2	27.4	6.9	73.6	85.7
42	30.3	23.7	27.0	23.3	82.4	92.4
43	30.2	23.5	26.9	7.1	80.9	93.6
44	30.5	23.5	27.0	4.8	86.1	85.1
45	30.7	23.1	26.9	1.0	93.1	73.4
46	31.2	23.7	27.5	4.4	90.4	74.3
47	29.4	23.4	26.4	9.4	95.9	79.1
48	29.1	23.1	26.1	8.3	93.6	84.1
49	30.6	22.6	26.6	5.1	90.1	67.9
50	29.9	23.3	26.6	24.3	89.6	77.0
51	30.6	23.4	27.0	4.9	93.6	78.9
52	29.9	23.8	26.9	6.0	90.9	75.4

Table 7. Weather data of 2015 at Vellayani

Standard	Temperature ^{0}C			Rainfall (mm)	RH %	
	Max.	Min.	Mean		max	min
1	30.5	22.2	26.3	4.0	95.1	67.7
2	30.4	21.9	26.2	0.0	91.1	63.9
3	30.8	21.8	26.3	0.0	95.4	64.6
4	30.6	21.6	26.1	4.0	92.6	65.0
5	32.0	22.4	27.2	0.0	92.1	64.3
6	31.6	23.2	27.4	0.0	94.4	60.7
7	31.1	22.5	26.8	0.0	93.0	61.9
8	31.2	21.0	26.1	0.0	90.3	70.1
9	32.1	23.3	27.7	1.0	88.7	66.6
10	32.1	23.3	27.7	0.0	88.6	66.3
11	32.1	23.6	27.8	15.2	91.4	69.3
12	32.7	23.3	28.0	0.0	90.7	67.9
13	33.0	24.7	28.9	9.4	90.7	67.0
14	33.1	25.2	29.2	2.3	91.9	70.0
15	32.6	24.3	28.4	4.8	91.4	68.6
16	32.9	24.3	28.6	40.4	89.7	76.5
17	32.5	23.8	28.1	35.5	89.6	77.3
18	33.2	25.2	29.2	0.0	85.1	75.9
19	32.5	25.2	28.8	34.4	91.4	83.6
20	30.4	24.3	27.4	29.3	94.0	89.1
21	32.3	26.1	29.2	19.5	92.1	82.7
22	31.9	25.2	28.5	6.5	90.8	81.0
23	31.9	24.7	28.3	12.3	89.7	79.6
24	31.9	24.0	27.9	10.5	91.7	83.9
25	31.6	24.4	28.0	6.8	90.3	82.7
26	30.5	24.0	27.2	26.9	92.0	86.6
27	31.6	25.3	28.5	2.5	90.1	79.6
28	31.9	25.2	28.5	5.1	88.1	80.9
29	30.6	23.8	27.2	7.0	90.1	81.1
30	31.3	24.1	27.7	3.2	87.9	76.9
31	31.3	24.5	27.9	2.3	87.6	78.1
32	31.8	24.7	28.3	1.1	90.0	76.1
33	32.4	24.5	28.5	14.4	87.9	73.4
34	31.8	24.7	28.2	5.3	91.3	76.7
35	31.9	24.7	28.3	0.0	89.9	81.1

36	31.5	24.2	27.8	20.2	91.7	84.3
37	31.2	24.0	27.6	13.5	93.4	86.4
38	31.0	24.6	27.8	16.5	93.1	81.9
39	31.8	24.5	28.1	13.8	88.9	83.0
40	31.2	23.9	27.6	5.8	91.9	79.0
41	31.3	23.8	27.6	21.3	92.6	80.6
42	31.4	24.4	27.9	5.2	91.1	78.9
43	31.2	24.2	27.7	8.5	93.3	82.4
44	31.1	23.5	27.3	29.2	92.7	83.1
45	31.6	24.1	27.8	6.1	93.1	79.4
46	31.5	23.6	27.5	13.1	92.1	81.7
47	32.0	24.0	28.0	13.3	94.1	76.9
48	31.7	23.8	27.7	19.2	92.6	80.7
49	31.1	24.2	27.6	7.1	95.0	85.7
50	31.8	23.9	27.9	42.1	94.9	89.7
51	31.1	23.9	27.5	7.2	95.7	78.7
52	32.2	22.9	27.5	0.0	92.8	72.3

Table 8. Weather data of 2016 at Vellayani

Standard weeks	Temperature			Rainfall (mm)	RH %	
	Max.	Min.	Mean		Max	Min
1	32.4	21.7	27.1	0.0	91.8	72.1
2	32.4	22.5	27.5	0.0	93.0	70.7
3	31.6	22.0	26.9	0.0	90.4	71.8
4	32.8	24.1	28.5	0.4	92.8	74.8
5	32.2	21.7	27.0	0.0	92.4	72.0
6	32.4	22.7	27.6	41.8	94.5	74.4
7	32.6	23.9	28.3	1.0	92.1	75.1
8	33.7	23.6	28.7	9.3	91.1	72.0
9	33.8	23.4	28.7	0	90.1	70.1
10	33.6	23.3	28.5	8.7	90.8	71.1
11	35.0	24.8	30.0	0	89.1	64.8
12	35.0	26.1	30.6	0	90.0	68.0
13	34.4	25.1	29.8	0	91.1	71.5
14	35.1	26.6	30.9	0.8	90.7	76.7
15	35.4	26.3	30.9	0.9	91.8	75.7
16	35.5	26.6	31.1	5.7	92.6	80.0
17	35.1	27.0	31.1	0	88.5	76.4
18	35.6	26.3	31.0	21.3	90.0	75.0
19	34.8	25.8	30.3	11.9	92.2	82.2
20	32.5	24.3	28.4	55.5	95.7	82.1
21	33.1	25.2	29.2	24.3	88.5	77.5
22	32.7	25.1	29.0	0	89.0	77.5
23	31.5	25.0	28.3	48.0	95.1	84.5

Table 9. 15 year mean weather data at Ambalavayal, Wayanad

Months	Temperature ^{0}C			RH %		Rainfall (mm)
	Min temp	Max temp	Average. temperature	max	min	
January	15.3	28.0	21.7	92.0	59.1	12.6
February	16.6	29.4	23.0	89.7	54.2	17.6
March	18.5	30.6	24.5	92.4	56.8	66.4
April	19.5	30.2	24.9	94.1	66.3	112.0
May	19.4	29.1	24.3	93.0	72.7	133.2
June	18.7	25.7	22.2	95.6	83.2	310.4
July	18.3	24.4	21.4	96.1	87.5	478.6
August	18.1	24.9	21.5	95.6	85.8	312.9
September	18.5	25.2	21.9	94.9	82.3	175.8
October	19.0	25.8	22.4	93.9	78.0	203.4
November	18.3	25.3	21.8	92.6	76.3	104.5
December	16.4	26.3	21.4	91.5	67.1	11.9

Table10. Chlorophyll content in sterile and fertile TGMS 03 plants (mg g^{-1})

Sterile			**Fertile**		
chl. a	Chl. b	Total chl	Chl.a	Chl.b	Total chl
1.48	0.93	2.41	0.62	0.27	0.90
0.77	0.25	1.02	0.56	1.01	1.02
0.46	0.13	0.59	0.70	0.77	1.41
0.77	0.27	1.04	0.46	0.84	0.93
Mean **0.87**	**0.39**	**1.26**	**0.58**	**0.72**	**1.06**

Table 11. Proline content in sterile and fertile TGMS 03 plants (μg g^{-1})

Sterile	Fertile	% Change
31.24	8.07	287.00
37.67	10.14	271.49
36.88	10.35	256.30
29.49	8.17	260.90
Mean **33.82**	**9.18**	**268.92**

Table 12. Total soluble protein content in sterile and fertile TGMS 03 plants (mg g^{-1})

Sterile	**Fertile**	**% Change**
14.88	6.98	113.18
19.78	7.72	156.21
13.79	6.20	122.41
21.81	5.14	324.32
Mean **17.56**	**6.51**	**179.03**

Table 13. Total free amino acid content in sterile and fertile TGMS 03 plants (mg g^{-1})

Sterile	**Fertile**
6.70	6.03
5.79	1.84
1.62	2.26
6.97	4.46
Mean **5.27**	**3.65**

Table 14.Malondialdehyde content in sterile and fertile TGMS 03 plants (µg g^{-1})

Sterile	Fertile
1.01	0.89
3.65	1.59
1.25	1.94
2.73	1.73
Mean **2.16**	**1.54**

Table 15. SOD activity (g^{-1}minute^{-1}) in sterile and fertile TGMS 03 plants

Sterile	Fertile	% Change
0.67	0.31	116.13
0.56	0.26	115.38
0.66	0.40	65.00
0.44	0.28	57.14
Mean **0.58**	**0.31**	**88.41**

Table 16. Ascorbic acid content in sterile and fertile TGMS 03 plants (mg g^{-100})

Sterile	Fertile
37.50	17.50
50.00	20.00
50.00	45.00
50.00	20.00
Mean **46.87**	**25.63**

Table 17.PhenoIic compounds in sterile and fertile TGMS 03 plants (µg g^{-1})

Sterile	**Fertile**
9.28	13.60
17.49	6.86
58.39	35.06
62.01	6.36
Mean **36.79**	**15.47**

able 18. Auxin content in sterile and fertile TGMS 03 plants (µg g^{-1})

Sterile	**Fertile**
0.25	0.23

Table 19. Photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) and transpiration rate (mmol H_2O m^{-2} s^{-1}) in rice.

Plants	Photosynthetic rate (µmol CO2 m-2 s-1)	Transpiration rate (m mol H2O m-2 s-1)
Uma	10.57	0.72
Jyothi	10.07	3.88
TGMS 03	7.57	2.21
F1 of Uma	2.20	0.46
F2 of Uma	1.70	4.81
F2 of Jyothi	4.70	2.17
F3 of Jyothi (TGMS)	39.40	0.47

a b

Plate 8. Male fertile anthers of Uma- unstained (a, b)

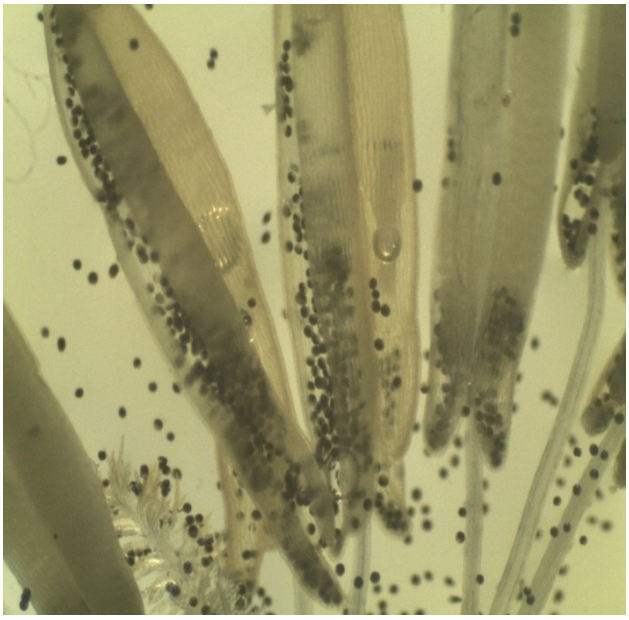

Plate 9. Male fertile anthers of Uma- stained with 1% IKI

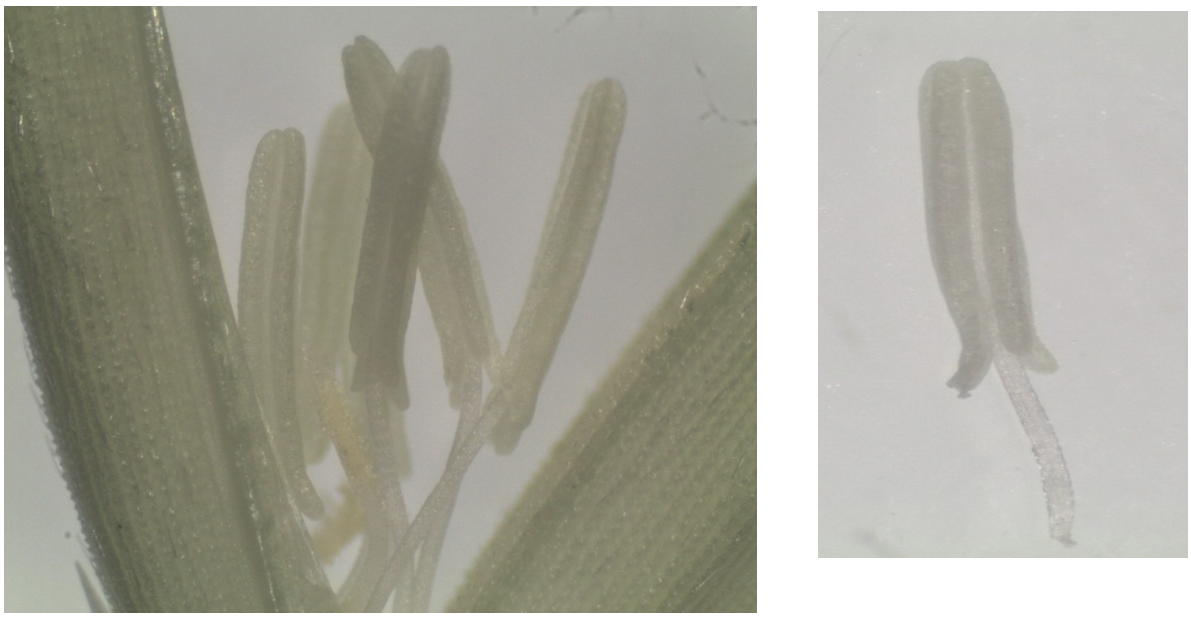

Plate 10. Male sterile anthers of TGMS 03

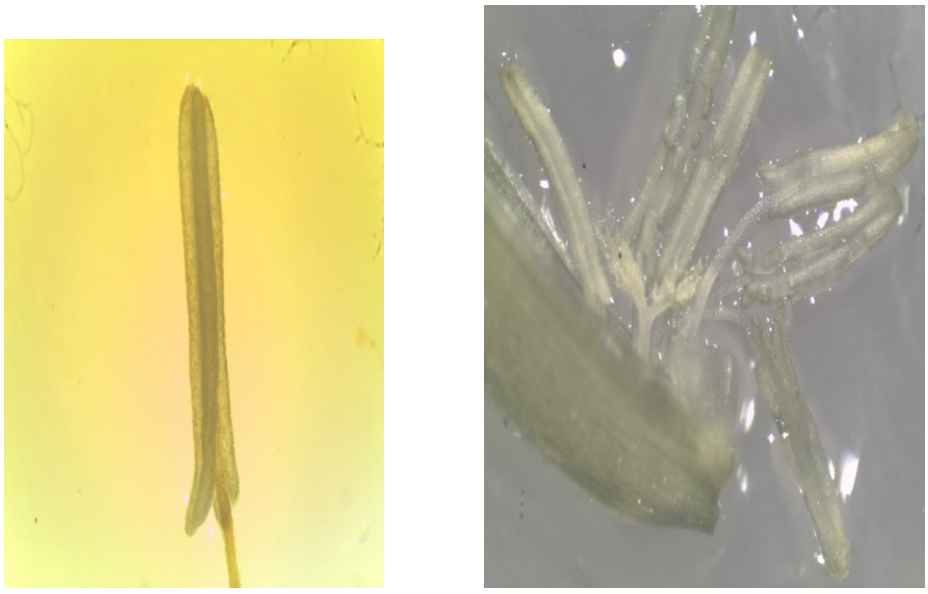

Plate 11. Male sterile anthers of TGMS 03- stained with 1% IKI

Plate 12. Male fertile and male sterile anthers of F_2 plant (03XJyothi)

Plate 13. Red male sterile F_3 seed on Jyothi background

Plate 14. Sterile/ pollen free anthers of TGMS F_3

Discussion

5. DISCUSSION

The prevailing situation in our state demands higher production of rice which can be achieved by exploiting hybrid vigour. Even though our country is having so many rice hybrids which are developed by three line system, utilising CMS, for a state like Kerala with a preference for red rice, TGMS system will be ideal. Kerala having clear temperature variation with altitude as well as seasons is suitability for utilising TGMS system. As TGMS trait is controlled by nuclear genes, it is transferrable and can be transferred to suitable red rice. In this context, the present work entitled "Physiological characterization of thermo-sensitive genic male sterility in rice (*Oryza sativa* L.)" was done with the objective to develop thermosensitive genic male sterile line in red rice background and to evaluate its physiological and phenological characters.

5.1 PHENOLOGICAL CHARACTERS AND PHYSIOLOGICAL CHARACTERS

According to Zhang and Zhu (1990) and Yuan *et al*. (1993), environment-sensitive male sterility- fertility system is a recessive trait. Ali *et al*. (1995) developed the tracking technique which was used in the present study to understand the CSP of TGMS line which was found to be 26.1 ^{0}C. According to Ali *et al*. (1995), the CSP and CFP is the factor which decides the usefulness of a commercial TGMS line. CSP of this new TGMS line was not known and hence evaluated. If it is very low, it affects seed multiplication and if it is high it affects hybrid seed production. So, the CSP of rice under the present study is found to be at desirable range in the point of view of commercial seed production. In the present study, as the TGMS is found to be male sterile with pollen free anthers, which is more stable male sterility than non pollen abortion, suitable in two line system of breeding according to Peng *et al.* (2010), is the most desirable character to be selected as the female parent as pointed out by Lakshmipraba and Thangaraj in 2005. As the TGMS plants set seeds at Ambalavayal having an average temperature less than 25 ^{0}C, it is an indication of better fertility reversion

which is also a favorable character for selection. Similar result was also reported by Lu *et al*. (1994). The pollen and spikelet sterility characters observed in this study is in line with the work of Matsui *et al*. (1997); Prasad *et al*. (2006), according to which high temperature lead to spikelet sterility due to poor anther dehiscence and low pollen production. According to Salgotra *et al.* (2012), there is need for identification of fertile and sterile sensitive phase of TGMS for different ecological areas, which will be helpful to determine the suitable location for hybridization and seed multiplication. In the present study, Vellayani condition was found to be the best for hybridization as the TGMS parent as TGMS F_2 plants were fully sterile all round the year. Seed multiplication of the same was successfully achieved at RARS Ambalavayal indicating that particular location was suitable for seed multiplication. This clearly shows that the temperature played a significant role in the fertility of TGMS line, which is in line with the report of Sanchez and Virmani (2005) and Ramakrishna *et al*. (2006). According to Lopez and Virmani (2000) in order to achieve seed multiplication at an economic level, seed setting of the TGMS plant should be at least 30 %, which in this study was found to be more than 30 %.

The present study shows that the TGMS line EC 720903 was male sterile at high temperature and was male fertile at lower temperature. The TGMS F_2 and F_3 plants (from red seeded F_2) obtained by crossing TGMS line with Jyothi parent also showed a similar nature with pollen free anthers as that of the female parent which is in line with the findings of Sreewongchai *et al.* (2014) where TGMS gene was successfully interrogated into Thai cultivar and had given sterile pollen at high temperature and fertile at lower temperature.

5.2 BIOCHEMICAL CHARACTERS

Rice being a staple food, its production as well as productivity is a matter of concern. The yield factors of rice mainly depend on its photosynthetic ability which points towards the chlorophyll content. The work revealed that the total chlorophyll content in leaves of sterile plants were higher than in the fertile ones

which is not in line with the results of rice under high temperature stress by Guhey *et al*. (2009). A similar result with a reduction in pigment composition was reported in rice by Sailaja *et al*. (2015). According to the work done by Gosavi *et al*. (2014), reduction in chlorophyll content was observed in maize during high temperature as well as water stress. According to Maisura *et al.* (2014), Chlorophyll b content is affected by the interaction of drought stress with variety and drought stress causes an increase in chlorophyll b. According to Sailaja *et al.* (2015), Chlorophyll b and carotenoid content were not affected significantly. Retention of chlorophyll for greater duration under high temperature was reported in tolerant genotypes of rice and creeping bent grass (*Agrostis palustris* Huds) (Sohn and Back, 2007). Chlorophyll b serves as an antenna that collects light and transfers to the reaction center chlorophyll a. Light energy is converted into chemical energy in the reaction center which can then be used in the reduction process of photosynthesis (Taiz and Zeiger, 2014). So the higher amount of chlorophyll in the present study may help for minimizing the photo-oxidative stress in sterile TGMS plants. The higher amount of chlorophyll can also be due to the absence of seed set in sterile plants.

Proline is an important amino acid that plants mainly accumulate during stress situations. A higher content of proline in sterile plants indicate its higher capacity to tolerate or survival in heat stress condition by acting as an osmo-protectant for maintaining the structure of cell (Kumar *et al.*, 2012), low molecular weight chaperon and protect enzymes and proteins. It also helps to maintain membrane integrity and scavenge ROS. During post stress period this proline can act as a reservoir of nitrogen and carbon (Parida *et al*., 2008; Hameed *et al*., 2012). Proline is synthesised in the cytoplasm during stress conditions and the same is inhibited during normal conditions. The result obtained is in line with that of Kumar *et al.*, (2012). An increase in proline content was also observed in sterile TGMS rice plants by Vijayalakshmi and Bangarusamy (2007).

Reduction in total soluble protein by subjecting plants to CST was reported by Vijayalakshmi and Bangarusamy (2007) leading to sterile pollen.

According to Noggle and Fritz (1986), major portion of soluble protein in leaves is occupied by RuBP carboxylase, so the measure of activity of enzyme is indirectly done by the estimation of soluble protein content. Vijayalakshmi and Bangarusamy (2007) also observed similar reduction in soluble protein content in sterile PGMS lines. Many authors (Peng and Wang, 1991; Huang, 1994; Stephen and Thangaraj, 2000) suggested the reduced availability of amino acids and reduced nitrogen assimilation for low soluble protein content which could cause male sterility in TGMS lines. These are not in line with the findings of the present study, where there is a much higher amount of total soluble proteins in sterile which may be due to a higher occurrence of Rubisco. This may also be connected with a higher amount of total free amino acids, and higher amount of phenols (which can act as a source of nitrogen) and a higher amount of chlorophyll. Male sterility may be due to the tapetal degradation as a result of PCD where specific TGMS genes may be involved.

In those TGMS rice plants which produced sterile pollens, Vijayalakshmi and Bangarusamy (2007) reported to observe about 55 % reduction in total free amino acids content which they suggested as the reasons for causing pollen sterility in plants treated at CST. Results similar to this was also reported by Peng and Wang (1991) and Stephen (1998). The outcome of the present work is not in convergence with the above data which may be due to the presence of higher amount of proteins, phenols (which can act as a source of nitrogen) and of chlorophyll in sterile plants.

Increased production of MDA during peroxidation of membrane lipids is often used as an indicator of oxidative damage. An increase in MDA content was noted by Sánchez-Reinoso *et al*. (2014) in rice grown at higher temperatures. The present work is in line with the same, where the sterile sample was having a higher MDA content. It thereby indicates a higher oxidative stress experienced by the sterile plants over fertile. Shah *et al*. (2011) reported an increase in the level of lipid peroxidation when the plant continues to grow under stress condition, whereby they confirmed the role of antioxidants as effective ROS scavengers,

leading to increased thermal stability by sustaining the integrity of membranes under high night temperature stress.

For scavenging ROS two types of anti-oxidant systems *i.e*, enzymatic and non-enzymatic are employed in plants (Hu *et al*., 2009). Increased SOD activity was observed in susceptible as well as in tolerant rice genotypes as reported by Sailaja *et al*. (2015) from the work done on rice. The biological role and significance of SOD as a protective enzyme against oxygen toxicity are reported in numerous higher plants (Bowler *et al*., 1992). The increase in SOD activity is induced as a defence to tolerate adverse environmental factors. From the work done by Alagarswamy *et al*. (2004) on TGMS rice SOD activity reduced drastically during CST treatment at different stages of panicle development in TGMS plants. Shen and Gao (1992) observed the same results in sterile PGMS lines under high temperature with long day conditions. Zhang *et al*. (1994a) also reported higher activity of SOD in fertile phase than in sterile phase of TGMS panicles, whose results were in line with the findings of Stephen (1998) who had suggested that higher temperature was an adversity for TGMS lines where it produced more super oxide radicals causing membrane damage and that this might be one of the most likely reason for temperature induced pollen sterility in TGMS lines. Zhang *et al*. (1994b) suggested that the low reduction potential of male sterile lines might have caused uncontrolled activated oxygen molecules and hence anther sterility. In this study the higher SOD content in sterile plants than in fertile plants may be due to the need for higher SOD content in sterile plants than in fertile plants as the former is having a much higher rate of membrane peroxidation which is evident from the higher amount of MDA in sterile plants. Even though the quantity of SOD is higher in sterile plants, it may not be enough to scavenge the ROS generated by membrane peroxidation which may be leading to male sterility.

The activity of AAO is a factor that determines the content of ascorbic acid and the AAO activity also varies in response to environment. The regulation of levels of oxidised glutathione, NADH and ascorbate content is the major role of AAO in plants. Lakshmipraba and Thangaraj (2000a) found that the activity of

AAO increased due to the induction of CST during the thermosensitive stage in the sterile TGMS lines. The study done by Alagarswamy and co workers in 2004 also revealed the constant reduction of AAO activity which was in accordance with the results of Liang *et al*. (1995) who reported an increase in ascorbic acid and glutathione content with reduced AAO activity in anthers under fertile condition. In the present study the ascorbate content was found to be much higher in sterile plants than in fertile plants, which may be due to the higher need for an antioxidant for scavenging ROS formed as a result of higher MDA, leading to male sterility.

Phenolic compounds are a diverse group of secondary metabolites and are widespread in plants (Waterman and Mole, 1994). Many plants respond to herbivory and other biotic or abiotic stresses by producing elevated levels of phenolics (Johnson *et al*., 1987; Pashley, 1988). An increase in phenol content was reported by Vijayalakshmi and Bangarusamy (2007) where in rice treated at CST, increased phenol content refered to the reason for the formation of sterile pollen in the variety TS 29. According to Rodionova *et al*. (1995), an association existed between phenolic compounds and long day and high temperature conditions that were adverse to normal functioning of plants. Result of the present work is in line with the findings of Stephen (1998), and Lakshmipraba and Thangaraj (2000a). The male sterility inducing property of phenolic compound coumarin was reported in Trigonella by Kaul and Singh (1967) and in *Vicia faba* by Awasthi and Dubey (1983). Salicylic acid is one of the phenolic compounds with thermogenic properties (Raskin, 1992). It also induced male sterility in rice (Lakshmipraba and Thangaraj, 1999; 2000b; 2004). The present work also reveals a higher amount of phenolic compounds in sterile plants which is in line with the findings of Johnson *et al*. (1987), Pashley (1988) as the higher amount of MDA, SOD, ascorbate in sterile plants clearly indicate the presence of stress experienced by these plants.

During reproductive phase of any crop plants, the activity of IAAO generally declines, so that the available auxin content can be utilised for the

flowering process (Paulas and Shanmugavelu, 1988). Hamdi *et al*. (1987) proved that the genes controlling maleness was correlated with auxin content and the sterility determinants modified the IAA content in male sterile line. Similar results were reported by Stephen and Thangaraj (2000) *i.e*, higher activity of IAAO depleted the auxin upto 33 % in sterile TGMS lines. Increased IAAO activity under sterile condition was also reported by He and Xiao (1993) in TGMS lines. These findings do not go in line with the results of the present work where there is a slight increase in IAA content in sterile plants which is very much comparable with that of the fertile. The possible reason for this may be the prevailing temperature during the period.

According to the findings from Sánchez-Reinoso *et al***.** (2014) an increase in the leaf photosynthetic rate of three Colombian rice cultivars was observed when the daytime temperature was increased from 25 to 35 °C. All rice cultivars exhibited significant reductions in photosynthesis at 40 °C. Transpiration plant rate (TPT) was enhanced by increased daytime temperatures (from 25 to 35 °C). When the daytime temperature was further increased from 35 to 40 °C, the TPT of these cultivars were decreased. Reports of Baker and Allen (1993), Wahid *et al*. (2007) and Stone (2000), the decrease in transpiration in some cultivars at 40 °C may be to prevent water loss. The increase in temperature from 25 to 35 °C also enhanced photosynthesis in each of the three Colombian rice cultivars. Similar result was obtained in the studies conducted by Taniyama *et al*. (1988) in *Indica* rice cultivars. Net photosynthetic rates of four rice cultivars improved when temperature increased from 25 to 35 °C, but were negatively affected at 40 °C. Cao *et al*. (2009) have suggested that high leaf photosynthesis under heat stress conditions might be a good indicator of heat stress tolerance. The results of the present study also shows a higher photosynthetic rate in the sterile TGMS F_3 plants developed from the red F_2 seeds which is in line with the findings of Cao *et al*. (2009).

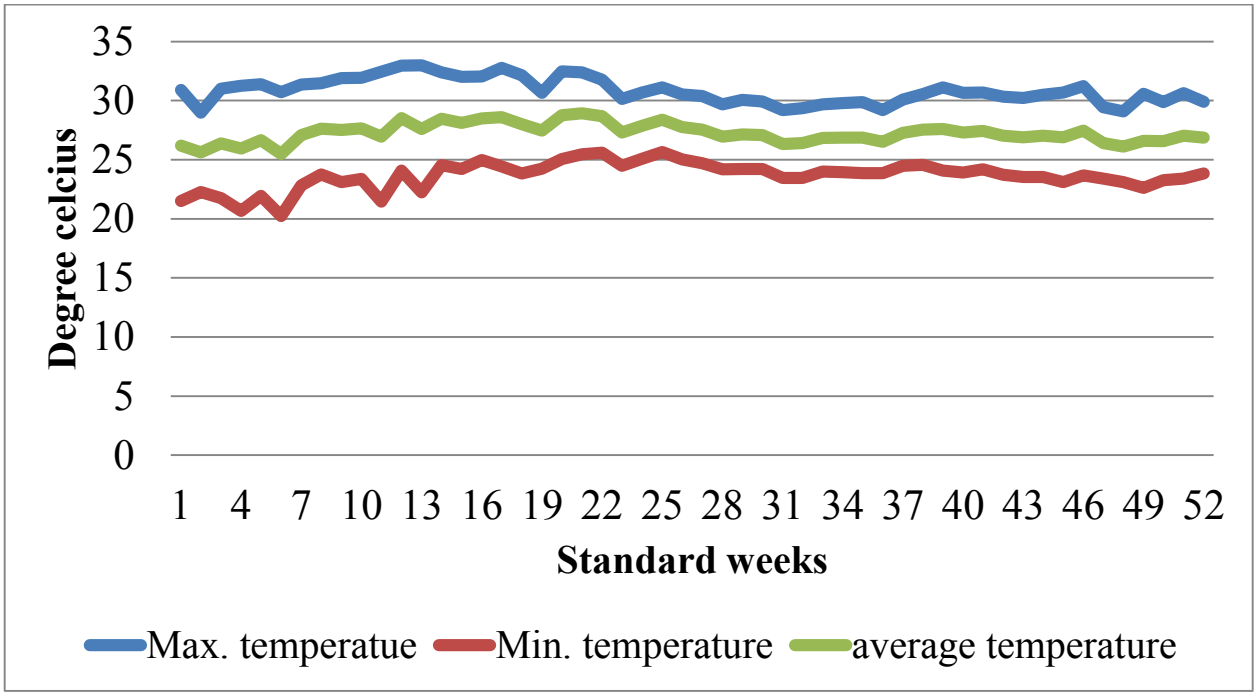

Fig. 1. Temperature variation at Vellayani during 2014

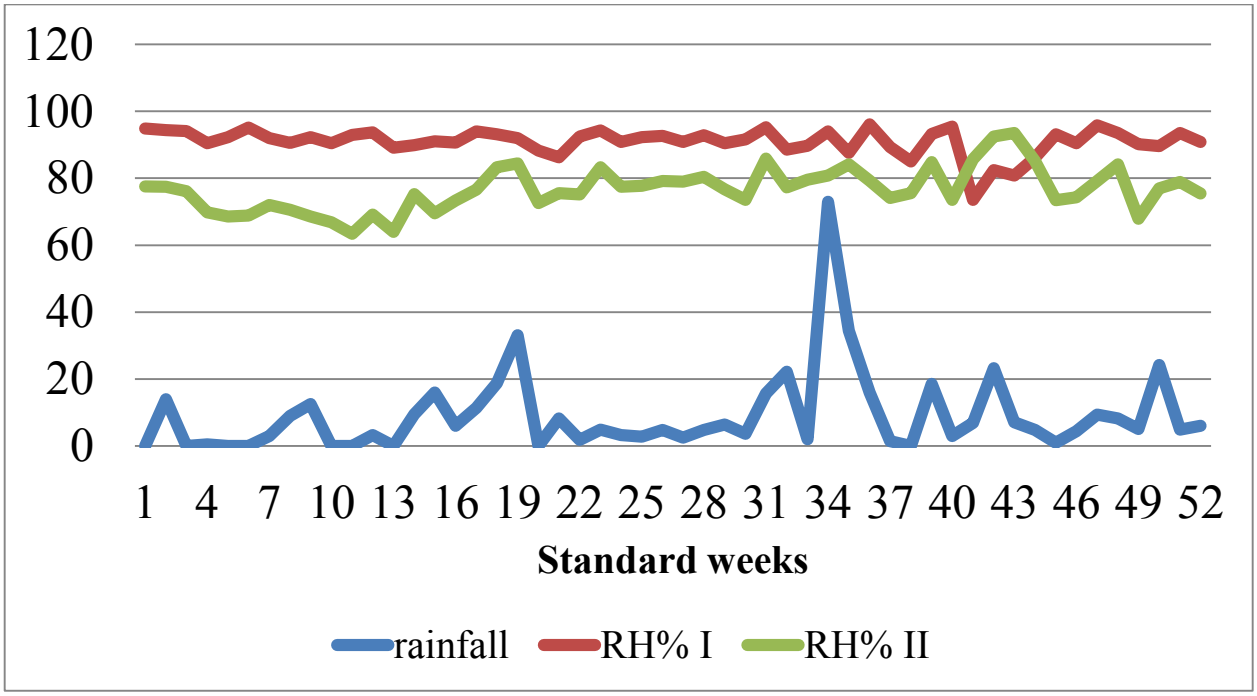

Fig. 2. RH and rainfall at Vellayani during 2014

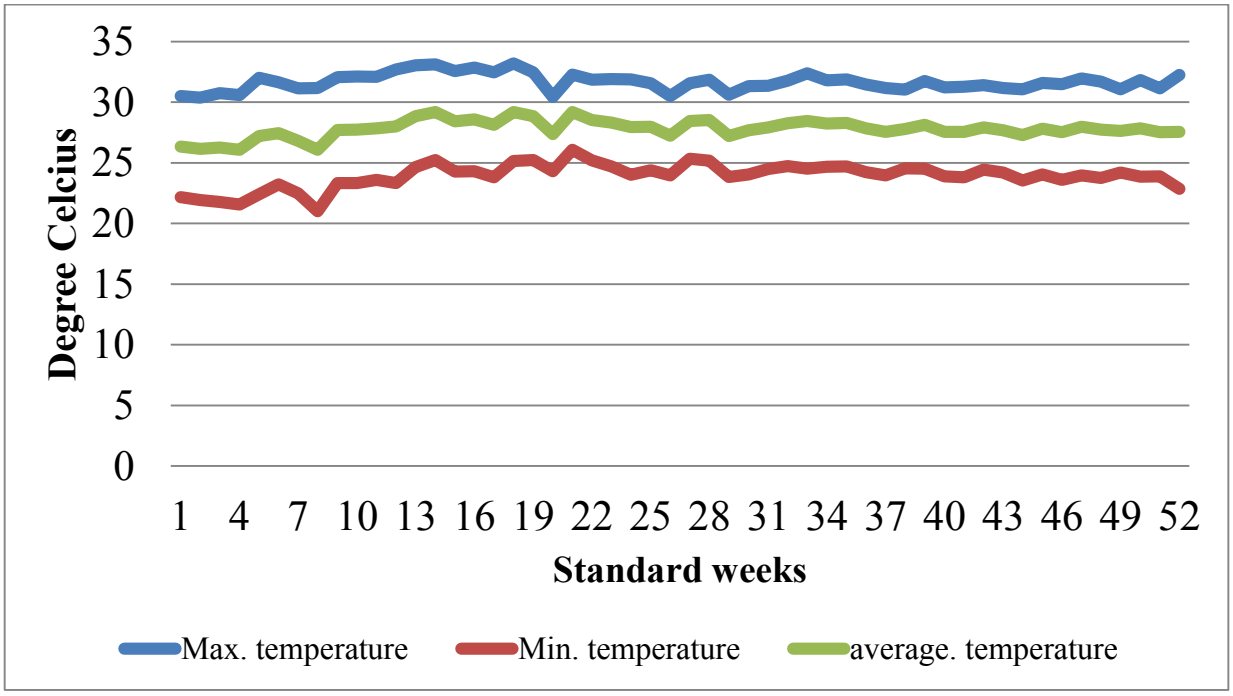

Fig. 3. Temperature variation at Vellayani during 2015

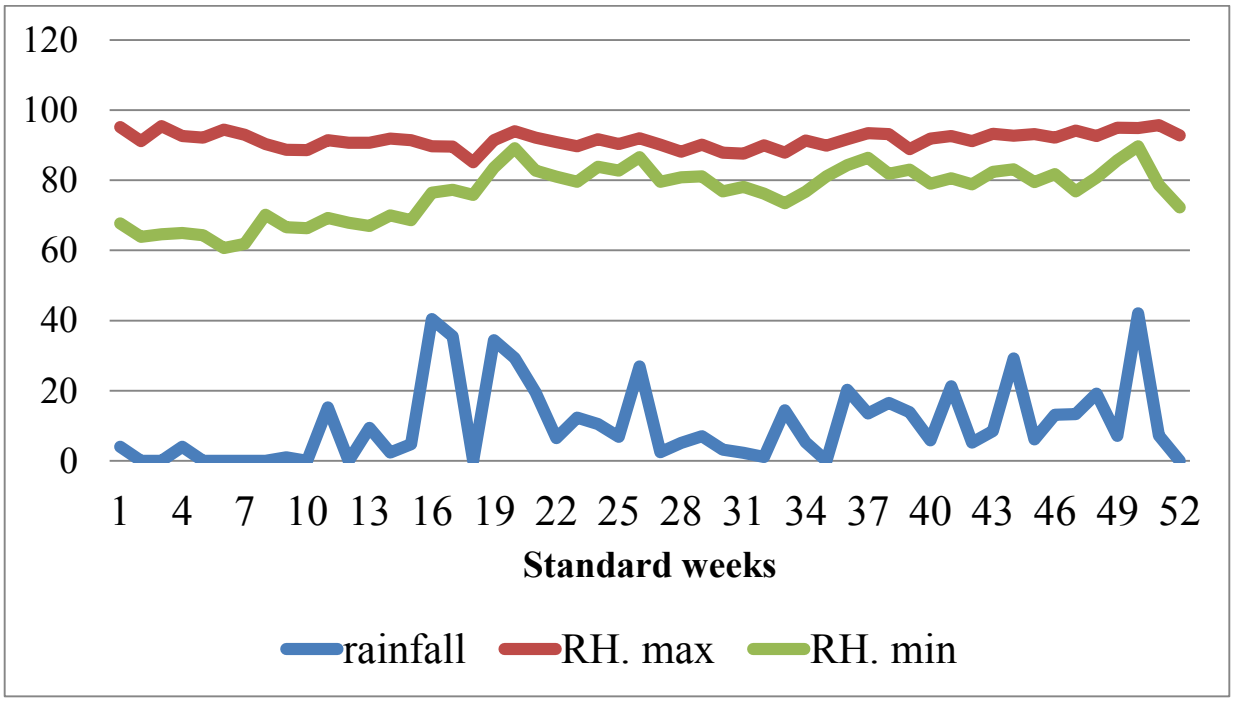

Fig. 4. RH and rainfall at Vellayani during 2015

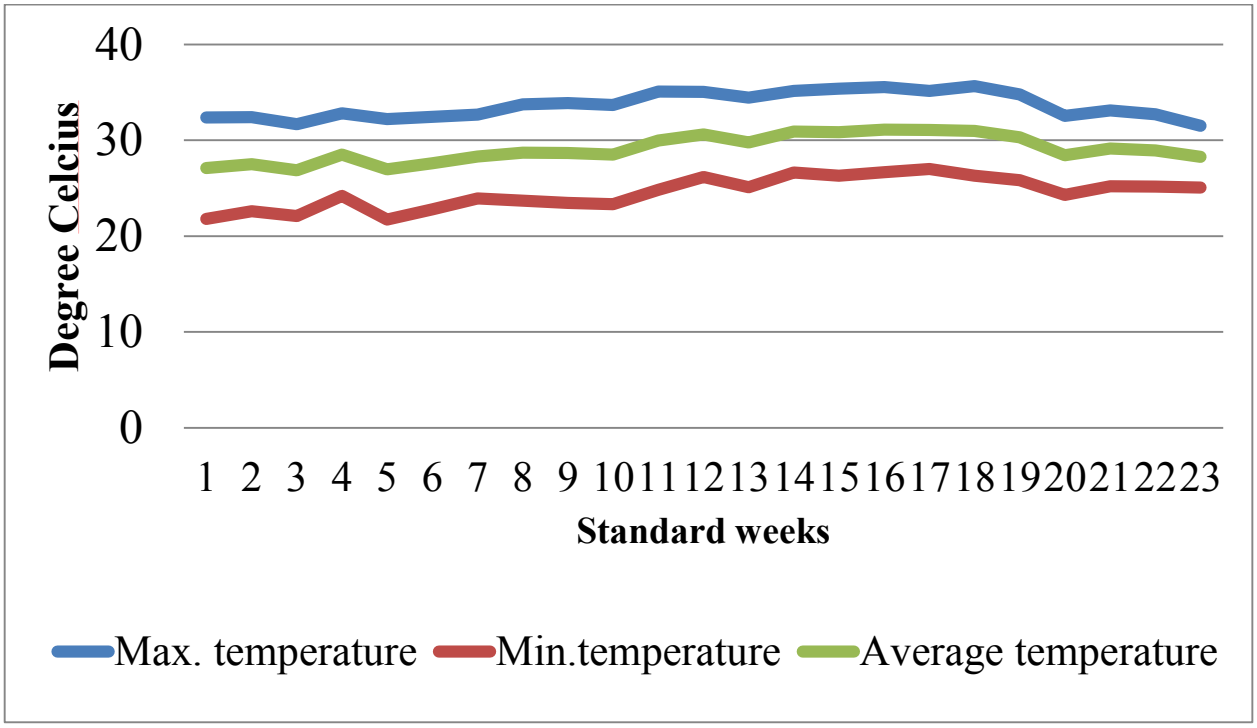

Fig. 5. Temperature variation at Vellayani during 2016

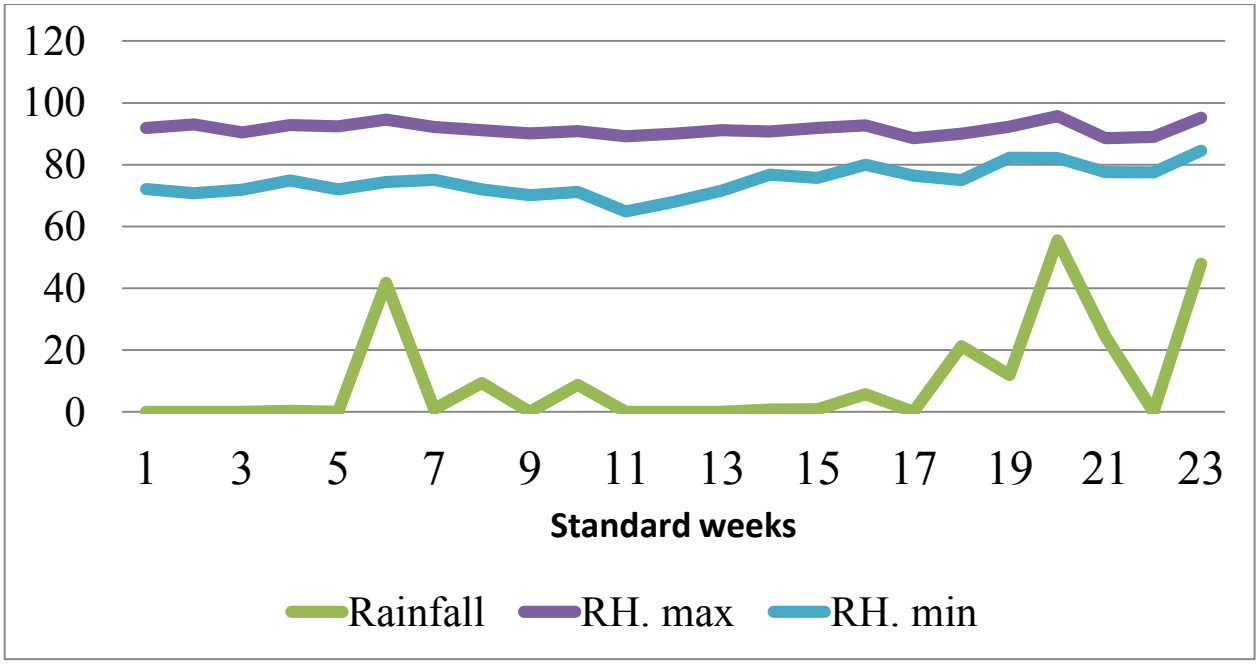

Fig. 6. Rainfall and RH at Vellayani during 2016

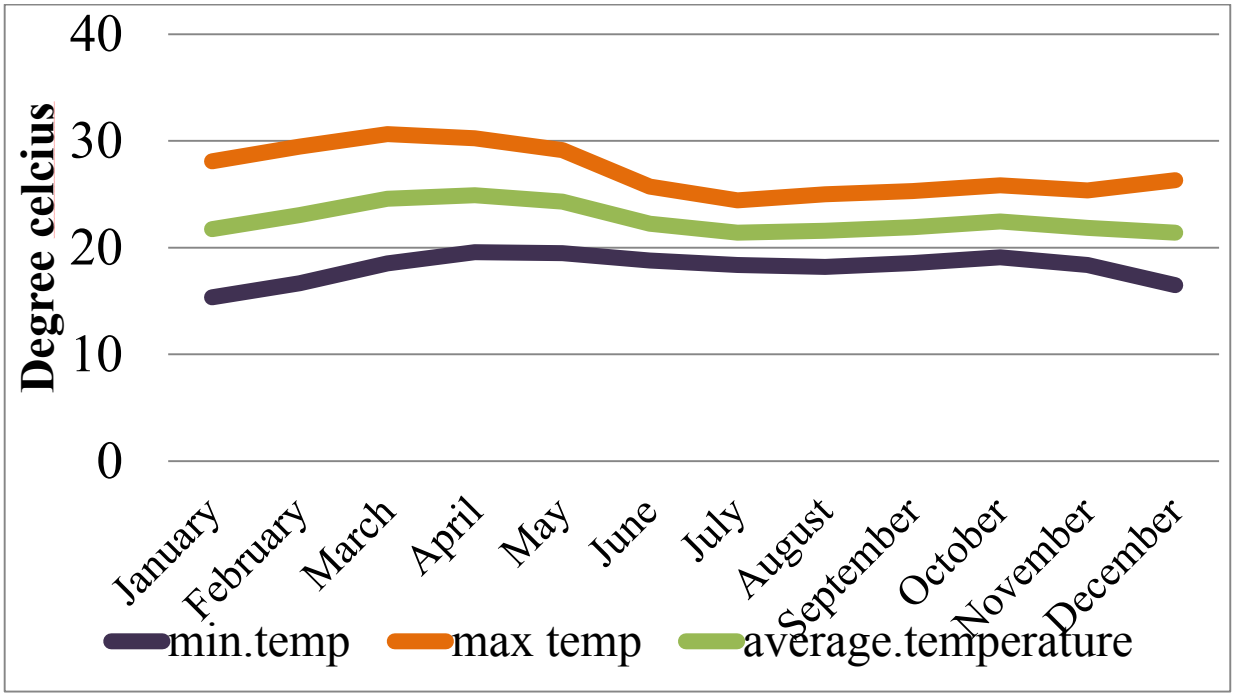

Fig. 7. Temperature variation at Ambalavayal

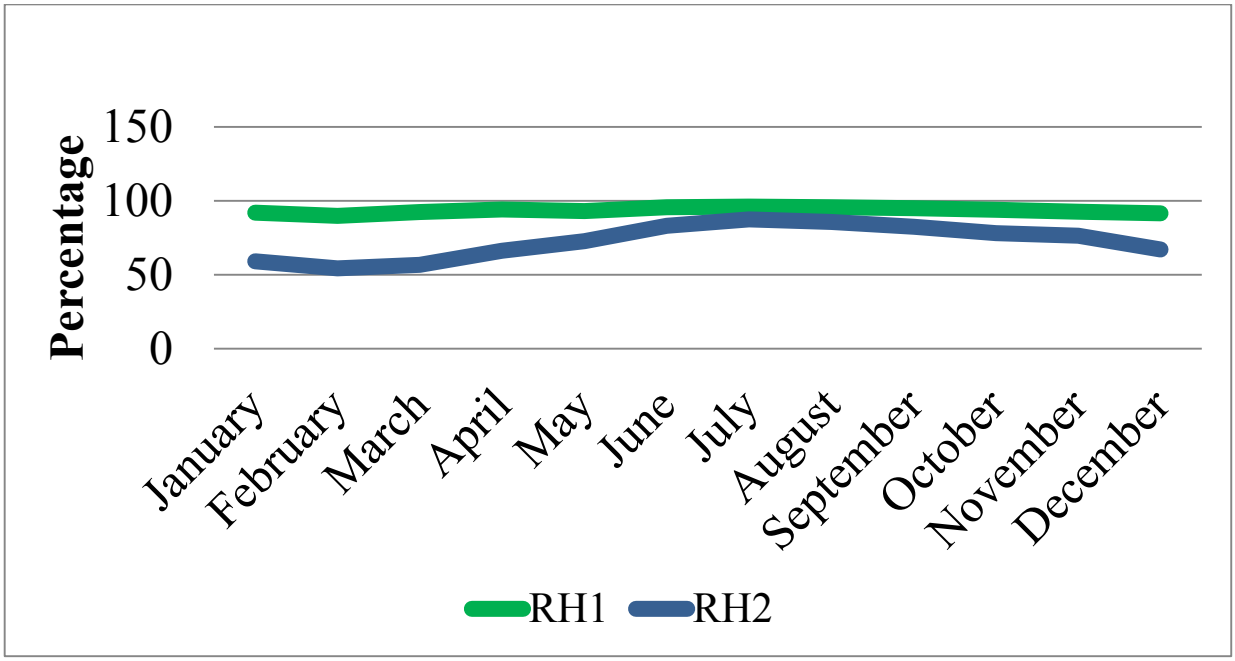

Fig. 8. RH at Ambalavayal

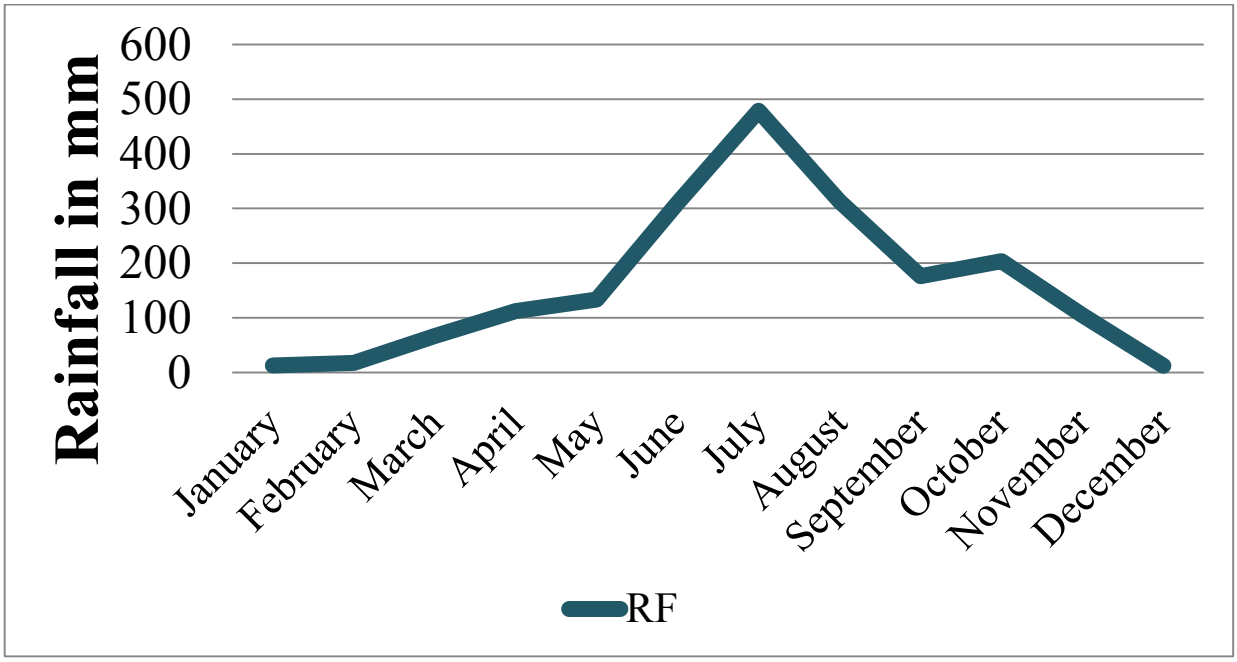

Fig. 9. Rainfall at Ambalavayal

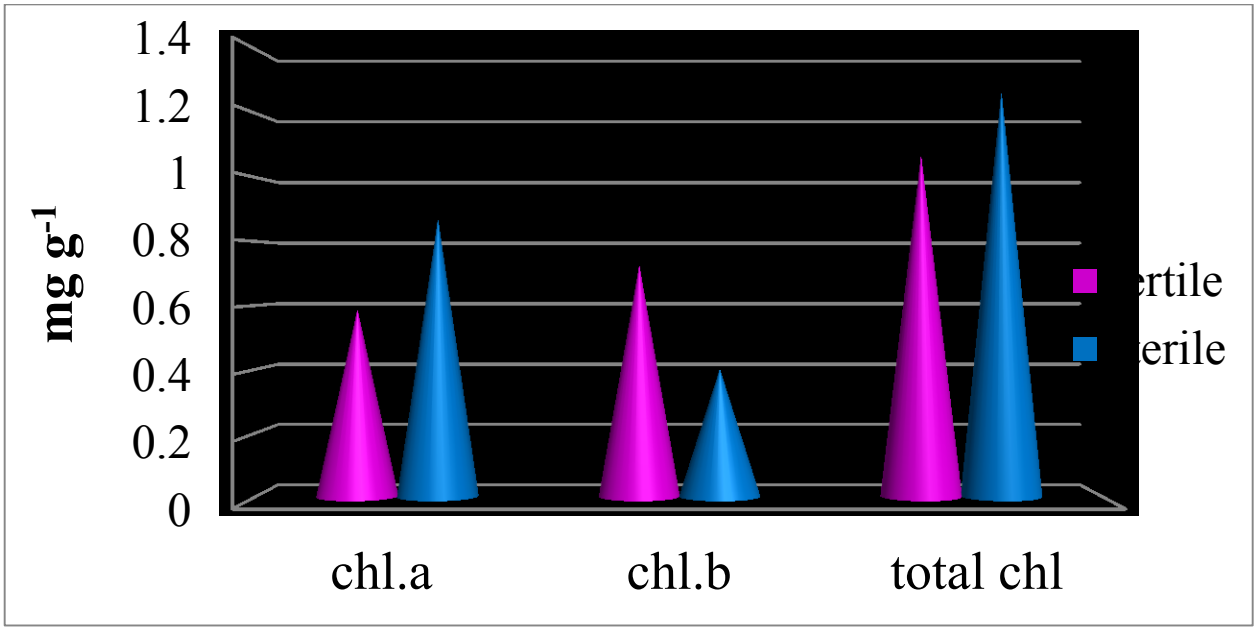

Fig. 10. chlorophyll content in sterile and fertile TGMS 03 plants

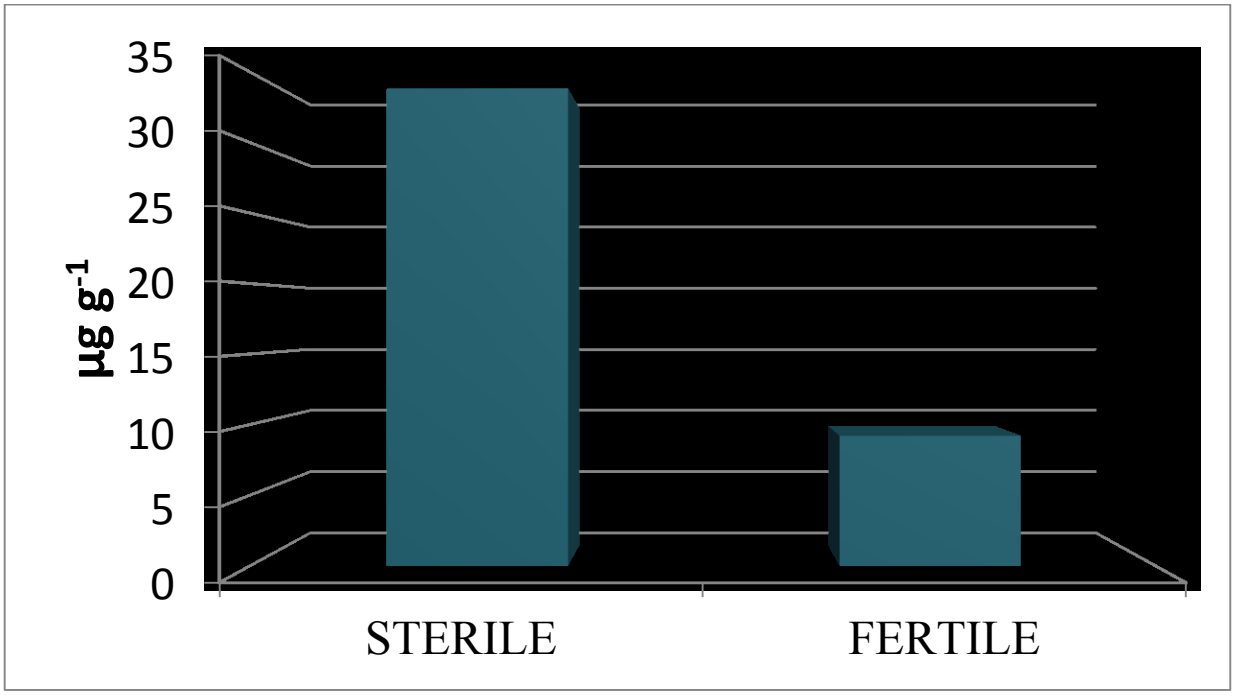

Fig. 11. Proline content in sterile and fertile TGMS 03 plants

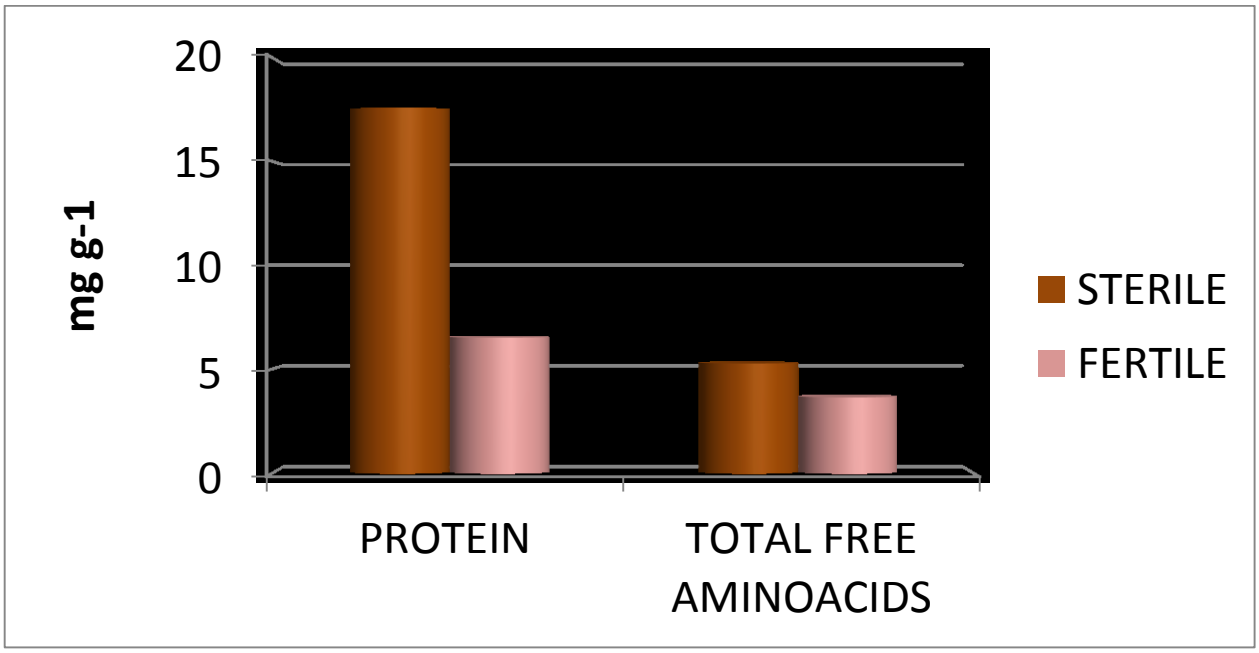

Fig. 12. Total free aminoacid and protein content in sterile and fertile TGMS 03 plants

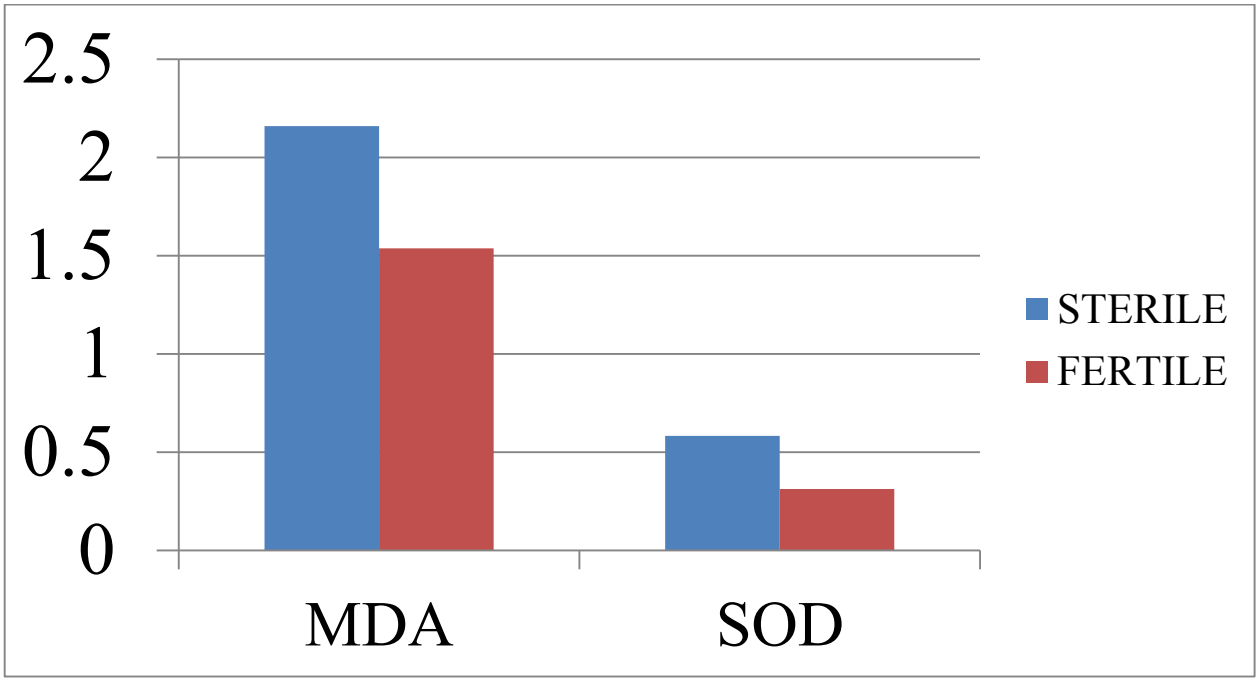

Fig. 13. SOD and MDA content in sterile and fertile TGMS 03 plants

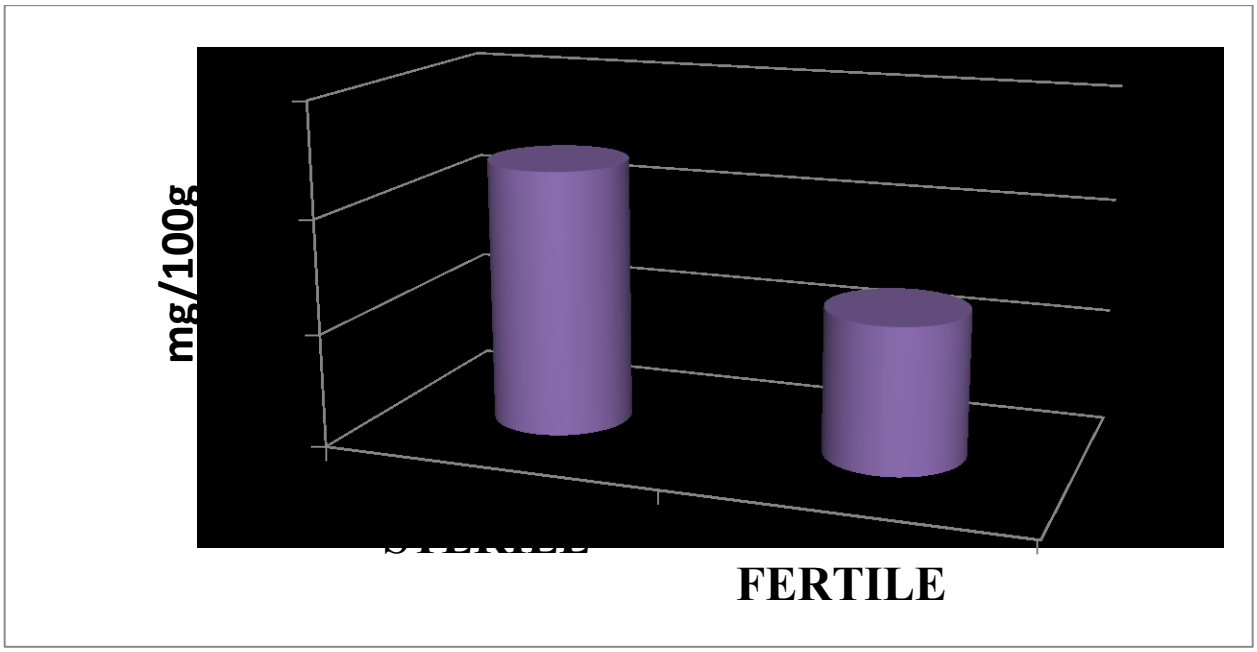

Fig. 14. Ascorbic acid content in sterile and fertile TGMS 03 plants

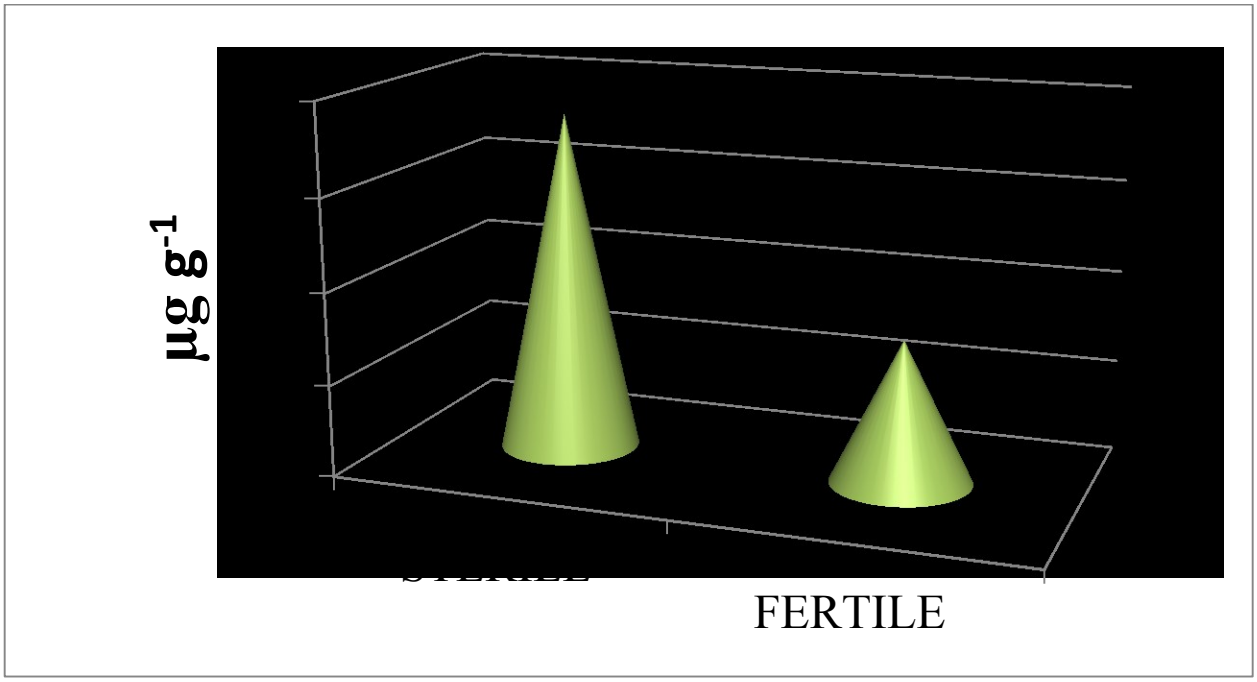

Fig. 15. Phenolic compounds in sterile and fertile TGMS 03 plants

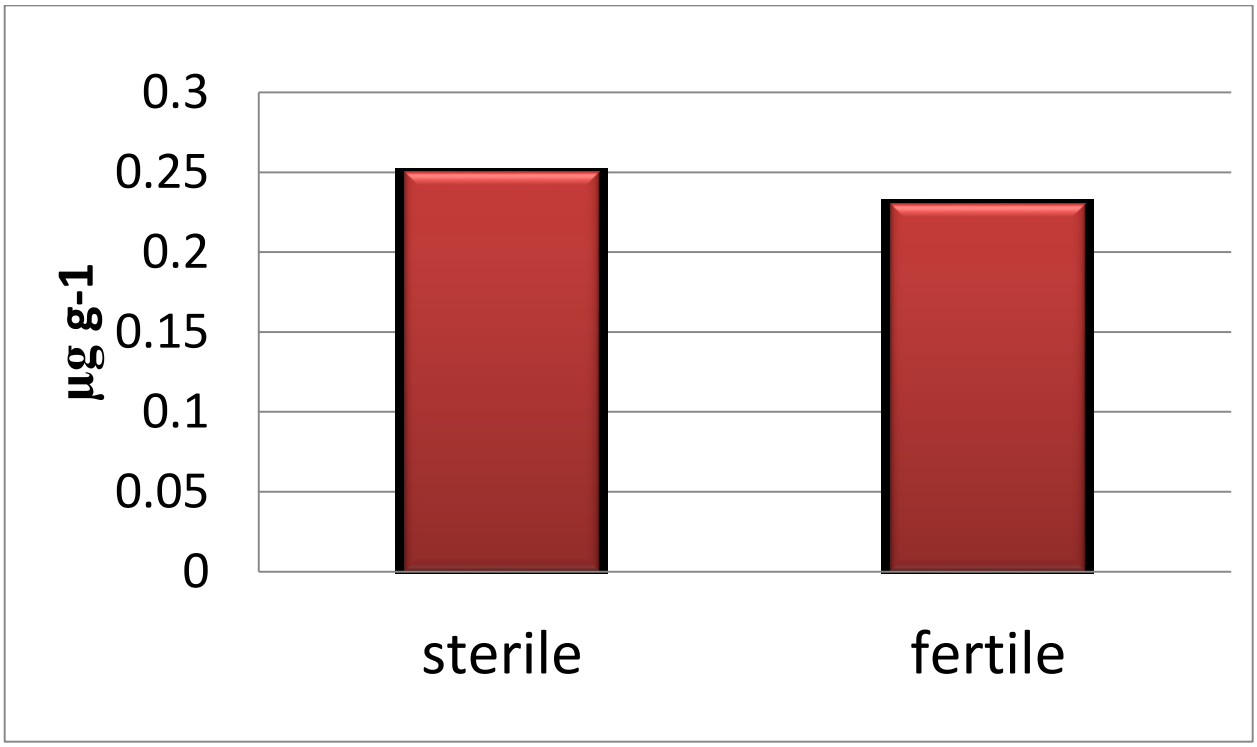

Fig. 16. Auxin content in sterile and fertile TGMS 03 plants (µg g^{-1})

Summary

6. SUMMARY

Rice is the staple food for a large population all over the world. For Keralites, rice is an inevitable cereal which they include in almost all the meals of a day. Unlike the other parts of the world Keralites generally prefer red rice which is bold in nature. This is one of the reasons for the acceptability of varieties such as Uma and Jyothi among Keralites. The area and production is not enough to meet the growing demand, which means there is a considerable demand supply gap. As the land availability is limited in a state like Kerala, in order to increase the production as well as productivity we have to adopt newer techniques which is suitable for our climate, topography and it should be equally acceptable for the rice cultivating farmers of our state. By considering all these aspects, the TGMS system is a very suitable one in our condition for producing hybrid rice seeds which is more productive than the conventional CMS system with an extra productivity of about 30%. TGMS line can be used for two line hybridization at plains and multiplication at higher altitude zone.

In this context the present work "Physiological characterization of thermo-sensitive genic male sterility in rice (*Oryza sativa* L.)" which attempted to develop a thermo-sensitive genic male sterile line in red rice background and its physiological and phonological characterization assumes great significance. It was envisaged to generate a TGMS line suitable for Kerala and will also help to understand the climatic specifications required for hybridization and seed multiplication and also to manipulate according to our requirement.

Red rice varieties, Uma, Jyothi, and the TGMS line from IRRI (EC720903), were the parental materials used in this study. They were maintained in pots. On flowering, the observations such as date of first flowering, date of 50% flowering, date of crossing, date of maturity, pollen sterility, spikelet sterility were taken. These plants were used for hybridization work using the TGMS line as the female parent.

F_1 seeds were collected, F_1 plants were raised and selfed to obtain the F_2 seeds. The F_2 seeds were also raised and evaluated for male sterility on flowering. They were also evaluated for the date of first flowering, 50 % flowering, pollen sterility and spikelet sterility. The total free amino acid content was also noted down. Those F_2 which were found male sterile at Vellayani condition were taken to RARS Ambalavayal in order to confirm the TGMS character and was also used for seed collection. Weather data of Vellayani was collected to understand the critical sterility point, that is the particular temperature above which the plant is sterile. Weather data of Ambalavayal for last 15 years was also used in order to understand the temperature requirement for the TGMS line to be fertile for seed multiplication. By maintaining the TGMS plants at sterility as well as fertility inducing conditions, leaf samples were collected and analysed for various biochemical parameters such as chlorophyll content, total soluble proteins, total free amino acids, MDA, SOD, proline, ascorbic acid, phenolic compounds, IAA, to understand the mechanism of male sterility in TGMS system.

The TGMS line was found to be a suitable female parent for Kerala in rice hybrid production as it exhibited synchronous flowering and 100% sterility with pollen free anthers at Vellayani condition. The TGMS F_2 plants exhibited similar flowering behavior as that of the female parent with pollen free anthers with 100% spikelet sterility. From the temperature data of Vellayani, critical sterility point was found to be 26.1^0C, and the mean temperature at Ambalavayal was found to be always less than 25^0 C which shows the reliability of RARS Ambalavayal for seed multiplication. The total free amino acid content in F_2 plants was higher in sterile plants than in fertile.

The biochemical analysis revealed a higher quantity of chlorophyll, total free amino acids, proline, phenolic compounds, superoxide dismutase activity, malondialdehyde, ascorbate, auxin, total soluble protein in sterile samples than in fertile plants.

6.1 CONCLUSION

The present investigation was carried out with the objective to develop thermosensitive genic male sterile line in red rice background and its physiological and phenological characterization. The study revealed that TGMS line was male sterile at Vellayani and was male fertile at RARS Ambalavayal. CSP of the TGMS line under study was found to be 26.1^0C. It can be used as a reliable female parent in rice breeding programme as the anthers are pollen free. TGMS red rice under Jyothi background was developed. The presence of higher amount of MDA, SOD ascorbate, phenol etc. in sterile plants clearly indicates a higher level of oxidative stress experienced by the TGMS plants under sterility inducing conditions leading to the expression of sterility.

6.2 FUTURE LINE OF WORK

India is having hybrid rice developed by CMS system and none of these varieties are red rice which is demanding in a state like Kerala. So by adopting the TGMS system we will be able to develop red hybrids suitable for Kerala condition. As Kerala is having a temperature demarcation with seasons as well as altitude it is possible to rely upon TGMS system to develop suitable rice for Kerala.

The present work has shown possibility of getting a TGMS red rice as well as the suitability of TGMS line EC 720903 as the female parent in hybridization programme and also synchronous nature of TGMS with the commercial varieties like Uma and Jyothi in flowering. It also confirms the possibility of utilizing high altitude places like Ambalavayal for seed multiplication in TGMS. It has also depicted the changes at biochemical level pointing towards oxidative stress. So, the future line of work should be in such a way to fix the red character in the red TGMS under Jyothi background which is already developed. This can be done by going for back crossing with Jyothi for about seven generations. As the TGMS is a recessive trait, it won't be

lost. After fixing the red into this TGMS the plant can be used for further hybrid rice breeding programmes.

The influence of weather parameters other than temperature, as well as in combination with temperature can be studied in future. Yield attributes, pest and disease susceptibility etc of this TGMS lines in Kerala, need to be studied.

According to this study, the mechanism leading to male sterility is found to be the higher oxidative stress experienced by the TGMS plants. This can be further investigated at molecular level pointing towards the genetic mechanism behind the fertility alteration. Attempts to understand and properly manipulate the TGMS system will surely lead to a better future of rice cultivation in Kerala, along with the development of scientific community that can last over generations.

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Physiological characterization of thermo-sensitive genic male sterility in rice (*Oryza sativa* L.)

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8. ABSTRACT

The study entitled 'Physiological characterization of thermo-sensitive genic male sterility in rice (*Oryza sativa* L.)' was carried out at College of Agriculture, Vellayani during 2014-16. The main objective of the study was to develop thermo-sensitive genic male sterile line in red rice background and its physiological and phenological characterization. The TGMS line from International Rice Research Institute (IRRI), EC720903 and red rice varieties, Uma and Jyothi were used in this experiment. TGMS plants become male sterile at high temperature and male fertile at low temperature. Seed multiplication was done at Regional Agricultural Research Station (RARS), Ambalavayal where the temperature is low. The work was done in three experiments.

Experiment I. Development and evaluation of male sterile F_2 plants. The TGMS line as well as Uma and Jyothi were grown in pots for hybridization. First flowering, 50% flowering, crossing and maturity dates were noted and F_1 seeds were obtained. F_2 seeds were collected pollen and spikelet sterility were recorded. The TGMS line was found to be a suitable female parent as it exhibited synchronous flowering and had 100% sterility with pollen free anthers at Vellayani conditions.

Experiment II. Physiological and phenological characterization of thermo-sensitive male sterile line. F_2 seeds developed were used for this experiment. Seeds were sown at monthly interval and the critical sterility point was identified. Pollen sterility and spikelet sterility of F_2 plants were monitored. Those plants which were sterile at Vellayani were taken to Ambalavayal for confirming the TGMS character in ratoon crop. The TGMS seeds were multiplied at RARS Ambalavayal where the season suitable for seed multiplication was noted. From these plants more than 200 red seeds were collected. The F_2 plants exhibited similar flowering duration as that of the TGMS parents. The sterile plants were showing sterility characters similar to that of the female parent (TGMS) with pollen free anthers and100% spikelet sterility. From the temperature data of

Vellayani, critical sterility point (CSP) was found to be 26.1^0 C above which the TGMS plants showed complete sterility. The total free amino acid content in F_2 plants was higher in sterile plants than in fertile. The weather data from Amblavayal clearly indicates the reliability for seed multiplication at Ambalavayal where the mean temperature is always less than 25^0 C.

Experiment III. Samples were collected from sterility inducing and fertility inducing conditions, simultaneously and analysed for various biochemical parameters like chlorophyll, total free amino acids, proline, phenolic compounds, Superoxidedismutase (SOD), Malondialdehyde (MDA), ascorbate content, auxin, and total soluble proteins. Chlorophyll was found to be higher in sterile plants than in fertile plants. Total free amino acids, proline, phenolic compounds, SOD, MDA, ascorbate content, auxin, and total soluble proteins were higher in sterile plants.

The study revealed that CSP of the TGMS line under study is 26.1^0 C. It can be used as a reliable female parent in rice breeding programme. TGMS red rice under Jyothi background was developed. The presence of higher amount of MDA, SOD, ascorbate, phenol etc. in sterile plants clearly indicates a higher level of oxidative stress experienced by TGMS plants under sterility inducing condition which may be leading to the expression of sterility.