

**THERMOSENSITIVE GENIC MALE STERILITY (TGMS) IN RICE
(*Oryza sativa* L.) : AN INVESTIGATION ON PHYSIOLOGICAL,
BIOCHEMICAL AND MOLECULAR MECHANISMS OF
STERILITY AND FERTILITY ALTERATIONS**

*Thesis submitted in part fulfillment of the requirements for the degree of
Doctor of Philosophy (Agriculture) in Crop Physiology
to the Tamil Nadu Agricultural University, Coimbatore.*

BY

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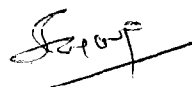
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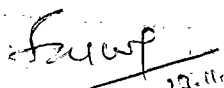
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Abstract

ABSTRACT

THERMOSENSITIVE GENIC MALE STERILITY (TGMS) IN RICE (*Oryza sativa* L.) : AN INVESTIGATION ON PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR MECHANISMS OF STERILITY AND FERTILITY ALTERATIONS

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A study was conducted at Tamil Nadu Agricultural University, Coimbatore during the year 1998-2001 to assess the critical sterility temperature and thermosensitive stage of TGMS lines and to investigate the physiological, biochemical and molecular bases of TGMS in rice. The possibility of chemical manipulation of fertility / sterility was also studied. The TGMS lines *viz.*, TGMS 6, TGMS 16 and TGMS 29 were used as test material along with ADT 36 for comparison with a normal variety.

Phytotron studies revealed that the CST of TGMS lines *viz.*, TGMS 6, TGMS 16 and TGMS 29 was 28.0, 27.0 and 27.5°C respectively and the critical thermosensitive

stage was 10-25 days before heading coinciding the secondary branch primordial differentiation to early meiosis of PMC.

The photosynthetic rate and hill reaction reduced under high temperature induced sterility due to reduction in Rubisco content and decrease in electron transport in PSII. The contents of nutrients Ca, Cu and B which play an important role in reproductive growth of plants reduced in sterile TGMS lines but the concentration of gaseous hormone, ethylene, increased under sterile condition.

The biochemical molecules such as soluble protein and total carbohydrate contents reduced under sterile condition but the accumulation of starch was more in sterile TGMS lines. Increased accumulation of proline and total phenols were also noticed under sterile condition. The content of free amino acids, ascorbic acid and the nucleic acids DNA and RNA were reduced in sterile TGMS lines compared to fertile lines. The enzymes NRase, MDH, GDH and acid phosphatase showed decreased activity whereas IAA oxidase and ascorbic acid oxidase activities increased under high temperature induced sterile condition. Increase in temperature adversely affected the activity of the free radical scavenging enzymes catalase, peroxidase and superoxide dismutase, which showed their strong association with male sterility.

The protein profiles of leaves and anthers, isozyme banding patterns of CAT, POX, SOD, EST and PPO showed distinct differences between fertile and sterile lines. Morphological and anatomical studies revealed the small, shrivelled and ill filled sterile anthers under high temperature condition.

Attempts for the induction of fertility / sterility indicated that the exogenous application of IAA (50 ppm) at PI stage increased pollen fertility in sterile TGMS lines

whereas salicylic acid (800 ppm) spray at PI stage caused complete male sterility in TGMS lines under unfavourable weather conditions. The analysis of biochemical changes associated with male sterility by salicylic acid treatment confirmed the changes in various macromolecules responsible for fertility / sterility transformation in TGMS lines.

Accumulation of activated oxygen species, higher activity of IAA oxidase which deplete the IAA content in plants and increase in proline and total phenol contents were the main cause for male sterility in TGMS lines. The present investigation revealed that during higher temperature condition some physiologically active compounds were produced and acted as signals or messenger molecules affecting other physiological and biochemical processes leading to pollen abortion and thus causing male sterility. The distinct differences in isozyme banding patterns and protein profiles of male sterile and fertile lines showed potential of using them as biochemical / molecular markers for screening TGMS trait in rice. Chemical manipulation of fertility / sterility experiments revealed the potential of salicylic acid in inducing male sterility under unfavourable weather conditions and the effect of IAA in increasing pollen fertility for seed multiplication in TGMS lines under high temperature condition.

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Abbreviations

ABBREVIATIONS

2, 4-D	:	2, 4-Dichlorophenoxy acetic acid
AAO	:	Ascorbic acid oxidase
APS	:	Ammonium persulfate
AVG	:	Aminoethoxy vinyl glycine
BR	:	Brassinosteroid
CAT	:	Catalase
CFP	:	Critical fertility point
CFT	:	Critical fertility temperature
CGMS	:	Cytoplasmic genic male sterility
CHA	:	Chemical hybridizing agent
CMS	:	Cytoplasmic male sterility
CSP	:	Critical sterility point
CST	:	Critical sterility temperature
DAPI	:	Days after panicle initiation
DBH	:	Days before heading
DCIP	:	Dichlorophenol indophenol
DNA	:	Deoxy ribonucleic acid
DRR	:	Directorate of rice research
E	:	Transpiration rate
EDTA	:	Ethylene diamine tetra acetic acid
EGMS	:	Environment sensitive genic male sterility
ERR	:	Ethylene release rate
EST	:	Esterase
F	:	Fertile
FCRD	:	Factorial completely randomized design
FW	:	Fresh weight
GA	:	Gibberellic acid
GC	:	Gas chromatography
GDH	:	Glutamate dehydrogenase
GMS	:	Genic male sterility
IAA	:	Indole-3 acetic acid
IAAO	:	Indole-3 acetic acid oxidase
I-KI	:	Iodo potassium iodide
IRRI	:	International rice research institute

kDa	: Kilo dalton
LD	: Long day
MDH	: Malate dehydrogenase
MH	: Maleic hydrazide
NAA	: Naphthyl acetic acid
NBT	: Nitro blue tetrazolium
NRase	: Nitrate reductase
OD	: Optical density
PAGE	: Polyacrylamide gel electrophoresis
PAR	: Photosynthetically active radiation
PGMS	: Photosensitive genic male sterility
PI	: Panicle initiation
PMC	: Pollen mother cell
P _n	: Photosynthetic rate
ppm	: Parts per million
POX	: Peroxidase
PPO	: Polyphenol oxidase
PSII	: Photo system II
PTGMS	: Photo thermosensitive genic male sterility
PVP	: Polyvinyl pyrrolidone
RAPD	: Restricted amplified polymorphic DNA
RBD	: Randomized block design
RH	: Relative humidity
RNA	: Ribonucleic acid
r _s	: Stomatal resistance
Rubisco	: Ribulose 1, 5 bis phosphate carboxylase / oxygenase
S	: Sterile
SA	: Salicylic acid
SD	: Short day
SDS	: Sodium dodecyl sulphate
SOD	: Superoxide dismutase
TBA	: Tri iodo benzoic acid
TCA	: Trichloro acetic acid
TEMED	: N, N, N', N' – tetramethyl ethylene diamine
TGMS	: Thermosensitive genic male sterility
WA	: Wild abortive

Introduction

CHAPTER I

INTRODUCTION

Rice is the premier food crop in the world. It is cultivated in about 148 million hectares which produce about 560 million tons of grains. It is the staple food providing about two-thirds of the calories for more than two billion people in humid and subhumid Asia and one-third of the calorie intake of the nearly one billion people in Africa and Latin America. Rice is also a major source of protein and carbohydrates for the masses of Asia. Since the mid-sixties, when the release of semidwarf varieties triggered the green revolution, rice production has increased at 2.4 per cent per year, faster than the rate of population growth. Achieving self-sufficiency in rice production and maintaining price stability are important political objectives in low-income countries because of the importance of this crop in providing national food security and generating employment and income for low-income people (Hossain, 1995). Most Asian countries have done remarkably well in meeting the food needs of the growing population over the last quarter of the century. However, the future poses even more challenging and ambitious tasks. The yield ceilings of rice varieties of the green revolution era must be lifted yet again, because by 2020 the world must produce 350 million tons more of rice than it produced in 1992, to meet the demand created by increasing populations and rising incomes. This production increase must be achieved on less land, with less labour, less water, and less pesticides, and it must be sustainable (Anonymous, 1993). Using hybrid rice is one of the strategies to meet this immense challenge (Virmani and Dedolph, 1993).

The hybrid technology in a strictly self pollinated crop like rice with a yield advantage of 2-3 t ha⁻¹ over the best conventional varieties is a major land mark in the history of rice breeding. The technology now being adopted in over 58 per cent of its rice area has helped China during the last 10 years to advance its rice production by

40 per cent. Using the improved parental lines especially the male sterile lines better adapted to tropical environment, India has planted commercial hybrids in 1994 and brought over 2 million hectares under hybrid rice by the turn of 20th century. In spite of their potential and promise, the conventional three-line hybrids are not without limitations. Of various weaknesses the narrow genetic base for cytoplasmic male sterility (CMS), over 95 per cent of the hybrids in cultivation and those under development are "wild abortive" cytoplasmic based, long cumbersome process of developing parental lines with desired agronomic features, need of maintainers for multiplication of A lines, utmost care to maintain the genetic purity of all the three lines and above all, the higher seedcost are important. These limitations have promoted to look for alternative approaches to exploit hybrid vigour.

One of the possible alternative is the two line breeding system, which comprises of environmental sensitive genic male sterility (EGMS) and chemical induction of male sterility (Ali, 1995). Two line hybrids evolved through EGMS are capable of producing 9-10 t ha⁻¹ which have a yield advantage of 10-20 per cent over the best existing hybrids (Yuan, 1996).

EGMS comprises of photosensitive genic male sterility (PGMS) which is based on the variation in day length and thermosensitive genic male sterility (TGMS) which is caused by high temperature. In tropics, TGMS system is more useful where day length differences are marginal and high and low temperature prevails on plains and high altitudes respectively. Hybrids developed by two line system are likely to show stronger heterosis because there are less restrictions in the choice of parents compared to CMS system. There is no need for maintainer line; hence procedure for the development of hybrid is simpler. Any genotype can be utilized as male parent in this system. This ensures greater

flexibility in the choice of parents in a hybrid combination. EGMS genes can be easily transferred to any rice line with desirable characteristics. Negative effects associated with sterility inducing cytoplasm are avoided. The seed production programme is simple and more efficient.

India, being a tropical country with significant temperature variation at different altitudes and different seasons, TGMS system is more suitable (Ilyas Ahmed, 1996) wherein the genotypes become male sterile at particular temperature and become fertile at some other temperature (Borkakati and Virmani, 1996). Several TGMS lines have been identified recently in India as well as at IRRI and the temperature ranges inducing sterility / fertility are being standardized (Ilyas Ahmed, 1996).

Though considerable information is available on the heterosis and combining ability of two line hybrids using TGMS, the change of fertility to sterility and vice-versa in TGMS lines should be quite clear and distinct. This insists the need of understanding the physiological, biochemical and molecular basis of male sterility caused by high temperature. Studying the physiological, biochemical and molecular mechanisms of fertility/sterility transformations of TGMS lines would open new frontiers for possible external application of some chemicals or metabolites in manipulating deviation in fertility status under unexpected weather conditions. In TGMS lines, a reduction in temperature or one or more rains during the sensitive phase will result in partial sterility of the lines, so chemical manipulation may help us to induce male sterility more efficiently. The plant will become completely male sterile with no chances for partial fertility

With this background, the present study was undertaken with the following objectives:

- ♦ To identify the critical sterility temperature (CST) / critical fertility temperature (CFT) and thermosensitive stage for sterility / fertility transformation for selected TGMS lines.
- ♦ To understand the physiological, biochemical and molecular mechanisms of TGMS in rice.
- ♦ To study the possibility of induction of sterility / fertility in selected TGMS lines using chemicals and growth regulators.
- ♦ To understand the physiological and biochemical basis for sterility induction by chemicals and growth regulators.
- ♦ To identify a reliable biochemical / molecular marker for rapid screening of TGMS lines.

Review of literature

CHAPTER II

REVIEW OF LITERATURE

Because of the potential of hybrid rice technology to increase rice production and productivity, many countries are currently working to exploit the benefits of this technology (Virmani *et al.*, 1996). Countries like India, with high labour land ratio are likely to have the potential demand for hybrid rice technology (Lin and Pingali, 1993). Efforts to develop and use this technology in India began in the 1970s and were systematized and intensified in 1989 with the launch of a mission oriented project. Within seven years, more than 12 hybrids from the public and private sectors were made available for commercial cultivation with a target to cover 2 million hectares under hybrid rice by 2000 AD (Siddiq, 1996). Taking this long range strategy of rice breeding into account scientists have identified an alternative to the existing complicated and cumbersome CMS system, called thermosensitive genic male sterility (TGMS) system (Maruyama *et al.*, 1991; Virmani and Voc, 1991) which uses genic male sterility that is influenced by temperature. TGMS lines could be best utilized where there are tropical high lands and tropical plains as in India (Ikehashi *et al.*, 1994).

This chapter reviews the current status of research on TGMS system for two line breeding and the physiological, biochemical and molecular mechanisms involved in their fertility alteration.

2.1. Two line breeding

The excessive dependence on single source of cytoplasmic male sterility (CMS) *via*, WA and the cumbersome process of seed production and parental line development warrant alternate approaches to exploit hybrid vigour in rice. Two line breeding is one such possibility, that emerged following the chance discovery of a

photoperiod-sensitive genic male sterile plant called Nongken 58S, in the *japonica* variety Nongken 58 by Prof. Shi Ming Song of China (Shi, 1981 and Shi and Deng, 1986). Two different approaches *viz.*, environmental genic male sterility (EGMS) and chemical induction of male sterility are being followed in two line breeding (Ilyas Ahmed, 1996). Rice genotypes, which can not only do self pollination and multiply itself, but also show complete pollen sterility in response to certain range of photoperiod or temperature, at a critical stage of development are described as dual purpose genic male sterile lines (Yuan, 1990). The lines which exhibit sterility/fertility with respect to photoperiod are called photoperiod sensitive genic male sterile (PGMS) lines and those respond to temperature are thermosensitive genic male sterile (TGMS) lines (Siddiq, 1996). In the development of hybrids only two lines, *viz.*, female (EGMS) line and a pollen donor are needed and hence is called two line breeding (Shen and Gao, 1992). The use of EGMS lines made the hybrid seed production simpler and cheaper. Other advantage includes higher heterosis since any genotype can be used as male parent, which need not possess restorer genes. The negative effects associated with the sterility inducing cytoplasm are also avoided. EGMS gene follows Mendelian inheritance mode and so most desirable varieties can be developed into EGMS lines through consecutive backcrossing with an EGMS source (Virmani *et al.*, 1996 and Ilyas Ahmed, 1996).

2.2. EGMS system

In EGMS, fertility / sterility alteration is mainly influenced by photoperiod and temperature. Cheng *et al.* (1996) classified EGMS lines into three categories based on their sensitivity to photoperiod or temperature.

♦ Photoperiod sensitive genic male sterility (PGMS)

PGMS line is sterile when the photoperiod (day length) exceeds 14 h and the same line becomes fertile if placed under less than 13 h of photoperiod.

♦ **Temperature sensitive genic male sterility (TGMS)**

TGMS line is sterile when temperature exceeds 32° C/24 °C (day / night) and the same line becomes fertile when the temperature is below 24 °C/18 °C (day / night).

♦ **Photothermo sensitive genic male sterility (PTGMS)**

PTGMS line is governed by the interaction of photoperiod and temperature. This group has been divided into two sub types. One is photo/thermosensitive i.e. male sterility is mainly induced by long day length and temperature serving as a subordinate factor. Other subtype is the thermo/photosensitive, where male sterility is mainly induced by high temperature, with long day length as a subordinate factor (Shen and Gao, 1992).

2.3. TGMS system

In TGMS lines, the expression of male sterile gene(s) is decided by the variation in temperature rather than photoperiod. TGMS can be utilized for tropical countries like India, where low and high temperature prevails in high altitudes and plains respectively.

Maruyama *et al.* (1991) reported that the thermosensitive genic male sterility is a wide spread phenomenon in nature and can be obtained in many ways. Some male sterile lines were spontaneous mutants, others were developed by irradiation breeding (Virmani, 1992) or were selected from filial generations following hybridization (Zhou *et al.*, 1991).

2.3.1. TGMS lines in various countries

The first isolated temperature sensitive male sterile line was Annong 1S, a spontaneous mutant in Hunan province of China. The change in the pollen sterility of this type was influenced by temperature variations (Yuan, 1990). The first TGMS mutant induced by 20 Kr gamma rays in Japanese rice variety Remei was obtained by

Maruyama *et al.* (1991). The male sterile mutant designated as H89-1 exhibited no seed set under 31/24°C, partial fertility under 28/21°C and complete fertility under 25/15°C (Maruyama *et al.*, 1991). Virmani (1992) reported that the pollen fertility in this mutant was not changed by variation in day length *viz.*, 15, 13.5 and 12 h and later it was designated as Norin PL12.

Virmani (1992) found that the TGMS line IR 32364-20-1-3-2B developed from an *indica* rice variety at IRRI showed sterility at 32/24°C but partial fertility at 27/21°C and complete fertility at 24/18°C in phytotron. In Fuji, the lines 5460S and R59TS were developed by Sun *et al.* (1989) and Yang and Wang (1990) respectively and the lines showed fertility under 27/22°C but sterility under 33/28°C.

Transfer of TGMS gene from Norin PL12 at IRRI to different genetic background resulted in different lines with TGMS character *viz.*, IR68945, IR68949, IR 70118, IR 68294 (Siddiq, 1996). A mutant MSM 65-2 with 100 per cent male sterility under 30/18°C and partial to complete fertility when minimum temperature was higher than 22°C was identified by Sathyanarayana *et al.* (1995). Lu *et al.* (1999) reported five new TGMS lines such as 6442S, FJS-2, K-1405S, 2-2S and GD-2S from various centres of rice research. These lines were exhibited 100 per cent sterility at daily mean temperature of above 28 °C.

2.3.2. TGMS lines developed in India (Table 1)

Rangaswamy (1992 and 1994) first reported six indigenous TGMS lines which was the first report on TGMS lines in 1992 and he also reported five more TGMS lines in 1994 from Coimbatore. Sathyanarayana *et al.* (1995) reported a new source of TGMS line in India among spontaneously occurring sterile plants from *indica* rice cultivar IET 100726 subsequently Ali *et al.* (1995a) isolated six TGMS lines *viz.*, SM3, SM5, F61,

Table 1. TGMS lines reported in India

TGMS line	Source
TGMS 1	Mutant from BG-850-1
TGMS 2	Mutant from BR-1728-26-1-1-5
TGMS 3	Mutant from IR 20-1579-1633-55-26-42
TGMS 4	Mutant from RP-2095-5-7-34
TGMS 5	Mutant from RP-2095-5-7-344
TGMS 6	Mutant from RP-2161-106-1-1
TGMS 7	BIC,F2 of IR 628292 A/O.nivara/IR62829 B
TGMS 8	Mutant From ASD 16
TGMS 9	Mutant From IR 50
TGMS 10	Mutant from C 12 <i>japonica</i> purple
TGMS 11	Mutant from C 12 <i>japonica</i> purple
SM 3	Variety Prana
SM 5	Germplam
JP 2	Breeding line of unknown cross
JP 8-IA12	Breeding line of unknown cross
SA 2 (43)	Sodium Azide induced mutant of IET 1-1990
F 61	Sodium Azide induced mutant of IET 11990
TGMS 16	Selection from Norin PL.12/IR 56 (IR 68945)
TGMS 18	Selection from Norin PL.12/IR 36 (IR 68945)
IC 10	Selection from Norin PL.12/IR 36 (IR 68945)
ID 2A	Selection from Norin PL.12/IR 36 (IR 68945)
IR 68948-12-3-7	Selection from Norin PL.12/IR47686-17-2
IR 68292	Selection from Norin PL.12/IR 47686-17-2
TGMS 15	Mutant of IR 32364-20-1-3-2

JP2, JP18-1A-12 and SA2 which remained sterile at high temperature and turned fertile at low temperature. Among these lines JP 18-1A-12, F61 and SA2 were found to be the most promising sources of stable sterility. Later, Ali *et al.* (1996) found that the Pusa variety J24A showed TGMS character. At Directorate of Rice Research (DRR), Hyderabad, four TGMS lines *viz.*, IRTG5, IRTG6, TGMS1, TGMS2 and ATG series were identified.

Reddy *et al.* (1996) reported a new TGMS line IC 10 from Norin PL 12/IR 36. In Coimbatore, Elsy and Rangaswamy (1996) evaluated promising TGMS lines and reported that TGMS 6, 9, 10, 16 and 18 showed sterility / fertility variation according to changes in temperature. Elsy (1997) found that the TGMS 16 and TGMS 18 remained pollen free with empty anther sacs in peak summer months. Saxena and Singh (1996) reported the TGMS nature of IR68292 and IR68945 from Pantnagar. Thiyagarajan *et al.* (1996) identified TGMS 15 and TGMS 16 as stable promising lines for hybrid seed production based on a study conducted simultaneously at Coimbatore (low altitude) and Gudalur (high altitude). Vijayakumar *et al.* (1996) reported the promising TGMS nature of IR68945-4-33-14, IR68948-12-3-7 and IR68949-11-5-31 from DRR, Hyderabad.

Malik *et al.* (1997) reported three traditional rice lines with TGMS nature and these lines were isolated from 70 traditional rice lines collected from hilly areas of Orissa. They showed complete pollen sterility at daily mean temperature of 27.5°C (32 / 23 °C). Saxena and Singh (1998) reported three promising sources of TGMS *viz.*, IR 68292-10-34-25, IR 68949-5-31-34 and IR 68945-33-4-14-7, which showed complete sterility when sown in February to May and partial fertility when sown in June and July. TGMS strain of a new TGMS rice, UPRI 95-140 was investigated by Rongbai and Pandey (1999) and they reported that complete pollen sterility was obtained at daily mean temperature above 27.1°C. Ali *et al.* (2000) reported two new TGMS lines, JP 24 A and

JP 24B, from WA based Pusa 33-16A line. These lines showed typical sterility point at 33.8°C (JP 24A) and 35.1°C (JP 24B).

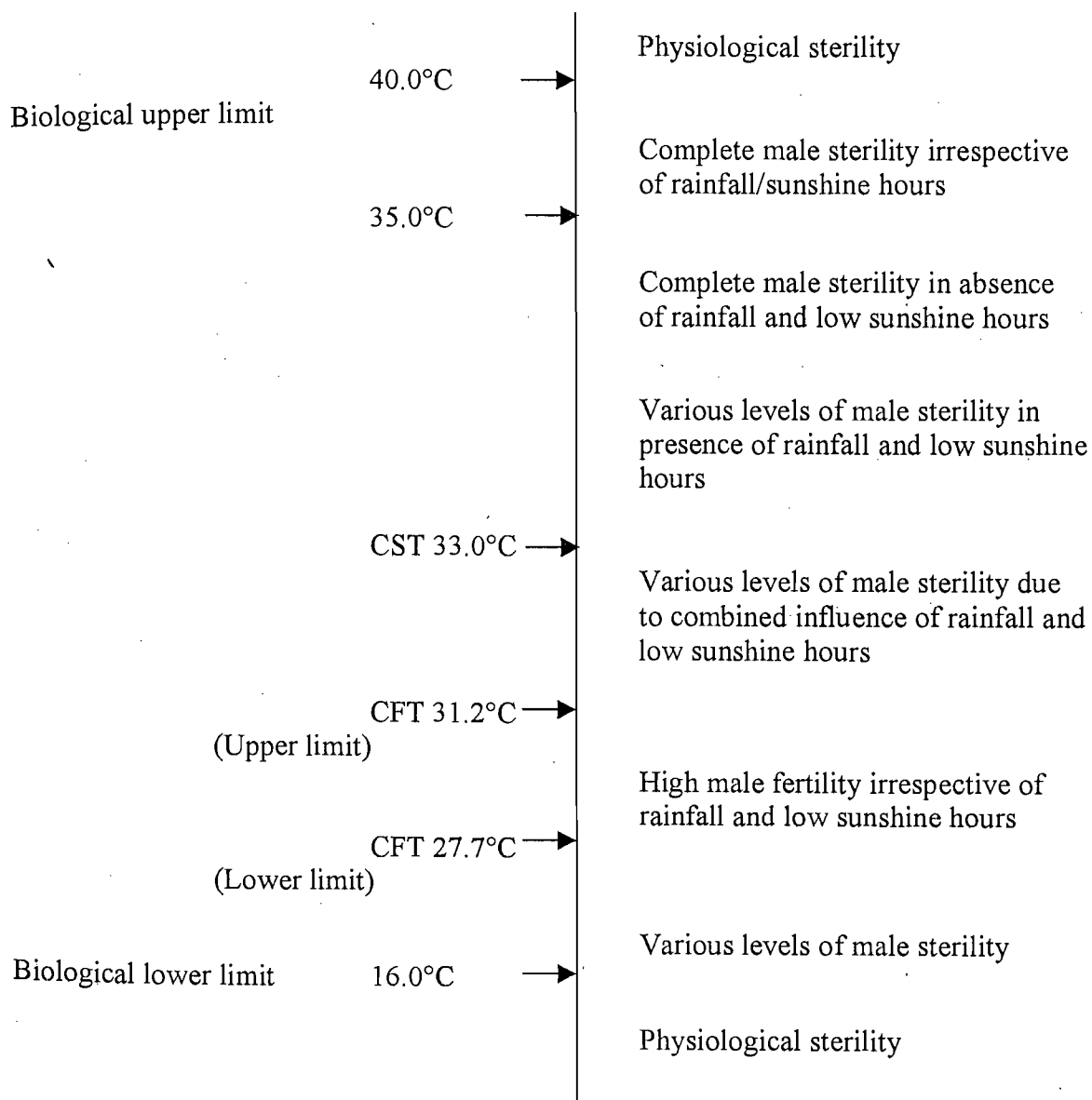
2.4. Factors influencing fertility transformation of TGMS lines

Virmani (1994) suggested that the stability and adaptability of TGMS lines across environments are the basic factors for their successful use in hybrid rice breeding. Yuan (1990) also reported the fertility of TGMS lines reacts differently to light and temperature when identical sterile lines were situated in different ecological environments. Thus, the external and internal factors causing the changes in fertility of TGMS rice are very complex and has diverted the attention and interest of the scientists all over the world working on hybrid rice seed production, in finding out the regularity.

2.4.1. External factors

Yuan (1990) reported that temperature is the major factor causing the change of sterility while day length plays a subordinate role in TGMS lines. Longer day length may induce sterility and shorter day length induces fertility when temperature is between critical value of sterility and fertility. Critical sterility point (CSP) is referred as the higher temperature at which complete sterility is induced and the temperature at which maximum fertility is achieved is called critical fertility point (CFP) (Yuan, 1990 and Ali, 1996). Zhang *et al.* (1993) reported that the CFP of a sterile line being used in hybrid seed production should be low enough, otherwise a low temperature may cause itself to self fertilize. If the CSP is not high enough it will be difficult to multiply the sterile line in higher temperature. These lines could be used for hybrid seed production and for multiplication during a specific season or at higher altitudes in tropical rice growing areas. The pattern of fertility change of TGMS lines in relation to temperature and day length is shown in Fig. a.

Fig. a. Pattern of fertility change in relation to temperature in TGMS lines



Yuan (1992) reported that the fertility change reaction to temperature and day length of TGMS lines under different genetic backgrounds was favourable for developing various types of TGMS lines suitable to different agroclimatic regions.

Yuan (1990) also suggested that the critical high temperature inducing sterility was 25-27°C and the critical low temperature inducing fertility was about 23°C in TGMS lines. The TGMS mutant, Norin PL12 and its derivative H89-1 were completely sterile at 31/24°C, partially fertile at 28/21°C and completely fertile at 25/18°C (Maruyama, *et al.*, 1991 and Zhang *et al.*, 1993). Cai, *et al.* (1991) reported that higher temperature of 35°C during PMC (pollen mother cell) meiosis stage i.e. 11 days before heading caused 100 per cent sterility irrespective of 12 h or 16 h day length in 5460S. At temperature of 25°C pollen fertility of all plants was resorted to normal, regardless of day length. A decrease of 4°C in temperature during light period increased pollen fertility, but a change during dark had no effect (Xue and Shen, 1991). The critical temperature for inducing male sterility varies from line to line from 23°C to 29°C (Yuan, 1992). For TGMS line 5460S, it was 29°C, which is not suitable for commercial use in the temperate zone or even in the subtropics, because even at 28.5°C, shift of male sterility to partial fertility occur.

The night temperature was more important in the expression of thermosensitivity than day temperature. The TGMS lines were sterile when maintained at 31/28°C and fertile at 24/22°C (Chen, *et al.*, 1993). A male sterile *indica* mutant 26 Zhaizao with a critical temperature of 23°C for fertility conversion under 12.5 h and 14.0 h day length was produced by Shen *et al.* (1994). Below this critical temperature the plants became completely male sterile. Zhang and Lu (1994) reported that the critical temperature varied with genetic background of the lines and the critical temperature for transformation from sterility to fertility in W61545 and W80135 was 24-29°C and for KS-14 and Pei ai 64S

was 21-24°C (Zhang *et al.*, 1994a). The critical temperature of Annong IS as daily mean of more than 26°C for stable sterility (Li *et al.*, 1995).

Yao *et al.* (1995) reported that the environmental control of fertility fluctuation of genic male sterile rice was due to the intensity and continuity of temperature and solar radiation. Borkakati and Virmani (1996) observed that the pollen of 7001S and E47S aborted completely under high temperature (30°C) and was completely sterile. The typical pollen abortion throughout and no pollen grains in the anthers during high temperature (30°C) were also shown by Annong 1S. Norin PL 12 showed high to complete pollen sterility at the maximum/ minimum range of 29.0-32.5°C/23-24.6°C. TGMS lines from IRRI viz. IR32364, IR68945, IR69294 were sterile at DRR Hyderabad, when flowered in June and July when the temperature was higher (max. above 30°C) and reverted to partial or complete fertility from August till February when maximum temperature was lower than 28°C. Reddy *et al.* (1996) reported that TGMS lines reverted back to fertility under cloudy weather and cooler night temperatures.

Thiyagarajan *et al.* (1996) evaluated TGMS 15 and TGMS 16 in Tamil Nadu simultaneously at Coimbatore (High temperature zone 38/23°C) and Gudalur (low temperature zone 30/16°C) during summer 1995. The lines showed 100 per cent pollen sterility at Coimbatore where as 55-80 per cent pollen fertility was observed at Gudalur. The TGMS line 68949-5-31-34 exhibited complete pollen and spikelet sterility when daily mean temperature was between 27.1°C and 28.4°C (Vijayakumar *et al.*, 1996). Lakshmi Praba and Thangaraj (1999) reported that TGMS 16 and TGMS 18 exhibited complete pollen sterility when the daily mean temperature was 26.5 and 27.5°C respectively. At these temperatures the lines were 100 per cent sterile. TGMS 16 was pollen free at this temperature. Roystephen and Thangaraj (2000) also reported the CST for TGMS 16, TGMS 18 and TGMS 29 as 26.5, 27.0 and 27.5°C respectively.

2.4.1.1. Classification of TGMS lines based on critical temperature

The understanding of critical temperature at which a given line will be male sterile or male fertile is important in commercial exploitation of TGMS lines in two line breeding for selecting the season or its multiplication or hybrid seed production. Based on critical sterility point (CSP) and critical fertility point (CFP), TGMS lines have been classified mainly into two groups (Ali *et al.*, 1995).

Group 1 High temperature sterility - Low temperature fertility

Type 1 : High CSP ($>32^{\circ}\text{C}$) low CFP ($<24^{\circ}\text{C}$) :

This type is recognized by Chinese scientists as ideal as it is safe for both hybrid seed production and maintenance. eg. SM3 and SM5.

Type 2 : High CSP ($>32^{\circ}\text{C}$) high CFP ($>24^{\circ}\text{C}$) :

This type is not suited for Chinese condition and it could be useful under tropical condition only. eg. JP-2, W7415S, 8902S and 7001S.

Type 3 : Low CSP ($<32^{\circ}\text{C}$) low CFP ($<24^{\circ}\text{C}$) :

This type is also ideal for tropical situation and could be used in hybrid seed production without any problem. CSP ranged from $30-32^{\circ}\text{C}$ and CFP from $20-24^{\circ}\text{C}$. eg. JP 8-1A-12, SA2 and F16.

Type 4 : Low CSP ($<32^{\circ}\text{C}$) high CFP ($>24^{\circ}$) :

Under this particular category lines are yet to be identified.

Group 2 : High temperature fertility - Low temperature sterility

In this type, the TGMS lines are reported to be sterile under low temperature ($22-24^{\circ}\text{C}$) and fertile under high temperature ($27-30.5^{\circ}\text{C}$). eg. Diaxin 1A and JP 38S.

2.4.1.2. Critical thermosensitive stage of TGMS lines

The critical stage or temperature sensitive phase of fertility alteration in TGMS lines is an important factor deciding the sowing time for hybrid seed production or seed

multiplication so that the critical temperature coincides with the critical thermosensitive stage. Yuan (1990) and Zhang *et al.* (1993) reported that the differentiation of secondary rachis branches and spikelet primordia to the formation of PMC was the sensitive phase in PGMS lines. Wang *et al.* (1991b) reported that the critical sensitive stage of Nongken 58S as that of stamen and pistil primordia differentiation and to a lesser extent that of panicle branch primordia and PMC meiosis.

The thermosensitive stage of Norin PL12 was around 22-26 days before heading for fertility transformation (Maruyama *et al.*, 1990). They found that even high temperature for an hour had adversely affected the seed fertility. Zeng (1991) found that the mean daily maximum temperature during 13-25 days before heading and mean daily minimum temperature during 10-18 days before heading were crucial in deciding the fertility transformation. Chen *et al.*, (1992) reported that the temperature during the meiosis stage of PMC was critical for fertility or sterility in plants.

In TGMS lines, Chen *et al.* (1993) reported that the stage most sensitive to temperature was gametogenesis to formation of PMC to the early monokaryon pollen stage. Zhang *et al.* (1994c) found that the thermosensitive period of fertility alteration occurred from the formation of PMC to the single nuclear pollen stage in W6154S. In TGMS lines, the sensitive stage was between 11 and 16 days before heading which coincided with PMC formation and meiosis in developing panicles (Zhang and Lu, 1994). Yang *et al.* (1995) found that the critical thermosensitive stage occurred about 20 days before heading and another at the meiosis stage in PTGMS lines. The sensitive stage varies according to the genotypes in TGMS lines. It was 17 days before heading in SA2, 19 days in JP-2 and F-61, 22 days in SM-3, 23 days in JPB-IA-12 and 24 days in SM-5 (Ali *et al.*, 1995). Yao *et al.* (1995) reported that the critical stage of thermosensitivity was 5 to 20 days before heading in TGMS lines. Lu *et al.* (1996) reported that the critical

stage of Norin PL 12 and IR32364 for fertility alteration was 6-15 days and 6-10 days respectively after panicle initiation. The critical stage of thermosensitivity for fertility alteration in TGMS lines TS 16 and TS 18 was 10 to 25 days before heading (Lakshmi Praba and Thangaraj (1999). Roystephen and Thangaraj (2000) reported that the critical thermosensitive stage of selected TGMS lines was 10-25 days before heading coinciding with secondary rachis branch primordia differentiation to early meiosis of pollen mother cells.

2.4.2. Genetic basis of TGMS

Male sterility expression in PGMS / TGMS lines is heritable but it is regulated by temperature or photoperiod. A single recessive gene in most of the crops reportedly governs EGMS. PGMS / TGMS traits in rice were controlled by single recessive gene (Shi 1985 and Sun *et al.*, 1989). Kaul (1988) reported that the pollen abortion in genic sterile lines is controlled by nuclear genes and is monogenic recessive. Male sterility genes can occur in nature through spontaneous mutations or be induced through physical and chemical mutagens. The TGMS trait in Norin PL 12 was controlled by a single recessive gene (Maruyama *et al.*, 1991). Kinoshita (1992) has registered the genes in 5460S and Norin PL 12 as tms1 and tms2 respectively.

Wu and Yin (1992) and Li *et al.*, (1995) the fertility expression in the stable TGMS lines Annong 1S and Hengnong S, was controlled by two recessive genes and were thought to be non-allelic. The TGMS gene on chromosome 8 was located using molecular markers in TGMS lines (Wang *et al.*, 1995a). Oard and Hu (1995) studied TGMS mutant M-201 and suggested that depending on the cross, pollen fertility was governed by one to three recessive nuclear genes. Subudhi *et al.* (1995) located the TGMS gene tms 3 (t) on short arm of chromosome 6 using molecular markers. Borkakati and Virmani (1996) studied the inheritance behaviour in Norin PL 12 and IR32364 and reported that a single

recessive gene controlled the TGMS trait in the two mutants. Allelic relationship studies indicated that TGMS gene of the two mutants were different. In IR 32364, it was designated as tms 3 (Lu *et al.*, 1996). Reddy *et al.* (1996) reported that though TGMS trait was governed by a single recessive gene, there might be some influence of modifier genes on the major gene affecting fertility. A study by Wu and Yin (1996) revealed that the variation in fertility expression in F₂ and back cross progeny from crosses involving 90336S with low sterility inducing temperature (SIT), 90332S and Annong 1S with medium SIT and 90341S with high SIT. Wu *et al.* (1996) concluded that the SIT differences between 90336S and 90332S was controlled by two independent dominant genes L₁ and L₂ and the SIT differences between Annong 1S and 90341S were controlled by an incompletely dominant gene H.

2.4.3. Internal factors

Bowler and Chua (1994) reported that the successful existence of all higher organisms is dependent upon their ability to co-ordinate complex developmental changes and to sense and respond to fluctuations in their surroundings. Responses to developmental and environmental cues occur by stimulus response coupling; a stimulus is perceived, a signal is generated and a physiological and biochemical change is instigated to cause the response. Thus the expression or suppression of TGMS gene depending on the external factors will alter the internal metabolism of the plant and is worth to understand the physiology during transition of fertility to sterility. This knowledge will be helpful in practically manipulating the sterility or fertility as needed by the breeders by inducing the same physiological or biochemical alteration.

2.4.3.1. Physiological changes during fertility alteration

2.4.3.1.1. Photosynthetic rate

Increasing temperatures lead first to a blockage of PSII reaction centres and then dissociation of antennae pigment protein complexes from the central core of the PSII light harvesting complex in photosynthetic system. (Gounaris *et al.*, 1984). At lower or higher temperatures carbon exchange rate (CER) was reduced in *Eragrostis tef*, a tropical grass, there by the net photosynthetic rate was affected (Kabede *et al.*, 1989). Synthesis of RuBP carboxylase and several thylakoid proteins responsible for photosynthetic electron transport and photophosphorylation was greatly inhibited at high temperatures (Maruyama *et al.*, 1990). Tong *et al.* (1992) studied the changes in photosynthetic activity of NK 58S and found that long day treatment decreased photochemical activity and efficiency of light energy transformation in PSII and also reduced the electron transmission rate of chloroplasts.

Xia *et al.* (1993) reported that reduction in ATP content and carboxylation rate under sterility could be associated with impaired photochemical efficiency or excitation energy transfer to the reaction centre protein complex. Tang *et al.* (1994) studied changes in leaves and anthers related to photoperiod regulation of fertility in LD and SD exposed Nongken 58S plants. Sterility inducing condition decreased photochemical activity and efficiency of light energy transformation in PSII and markedly reduced the electron transmission of chloroplasts. Chloroplasts from Nongken 58S in long day condition contained less chlorophyll 'b' than chloroplast from SD exposed plants. It was suggested that the decrease in photochemical activity in Nongken 58S chloroplasts during sterility might have decreased the concentration of photosynthetic products and affected male sterility.

Liang *et al.* (1995) reported that the respiration rate in mitochondria and lipoxygenase activity in anthers was lower in sterile lines than in fertile ones by 11.5 and 5 per cent at early uninucleate pollen stage and by 18.4 and 17.3 per cent at late uninucleate state respectively. Bose and Ghosh (1995) studied the high temperature effect on photosynthetic rate in rice and reported that the relative decline of the rate of whole chain electron transport and PSII activity was more pronounced. Soluble protein, chlorophyll content and RuBP carboxylase activity were relatively lower in leaves of male sterile line Pei ai 64S during sterile condition (Wang and Weicheng, 1996).

High temperature during flowering resulted in increased pollen sterility with the degree of sterility exacerbated if rice was exposed to both high temperature and increased CO₂ concentrations. Ma Dehua *et al.* (1999) studied the effect of high temperature stress on cucumber seedlings and reported that the chlorophyll content especially chlorophyll a, net photo synthetic rate, stomatal conductance and carboxylation efficiency were markedly reduced.

2.4.3.1.3. Hormonal Changes

Zhao *et al.* (1996) reported that indole acetic acid (IAA) deficiency in reproductive organs at an early developmental stages and reduction of GAs in later developmental stages were the factors causing anther sterility in Nongken 58S under long day condition. You *et al.* (1997) studied the endogenous hormone levels in male sterile lines and reported that the level of male sterility is related to the loss of endogenous hormones especially IAA. Roystephen and Thangaraj (2000) also reported that the major cause for male sterility might be due to the reduction of IAA levels by higher activity of IAA oxidase in TGMS lines.

High temperature treatment (40/27°C D/N) reduced the IAA levels and particularly auxin transport capacity in reproductive organs (Huberman *et al.*, 1997). IAA content increased during anther development of both hybrids and their parents (*Indica* x *Japonica*) but the contents were lower in sterile anthers than the fertile anthers of parents Nanjing 1 and Qui guang respectively (Zhu and Cao, 1997).

Luo *et al.* (1993) studied the relationship between ethylene metabolism and fertility change in NK 58S. Ethylene release rate (ERR) was higher under sterile condition than in fertile condition in young panicles.

Li DeHong *et al.* (1996) reported that the ERR was negatively correlated with pollen fertility and was low in long day low temperature (25°C) and high under short day high temperature (32°C). Treatment with aminoethoxy vinyl glycine (AVG) an inhibitor of ethylene biosynthesis, resulted in a marked increase in pollen fertility in male sterile plants. This indicated the key role of ethylene in the process of pollen sterility. Tian ChangEn (2000) studied the ethylene release rate in leaves and panicles and reported that the excess release of ethylene might be a cause of CMS in rice.

2.4.3.1.3. Nutrients

Boron, copper and molybdenum deficiencies induce male sterility in many cereals and other higher plants (Graham, 1975; Agarwala *et al.*, 1979; Dell, 1981). Crop plants subjected to micro nutrient deficiency exhibit reduction in anther size and pollen abortion. The pollen grains are poorly developed, small, shrivelled, highly vacuolated and lack of proper exine and cytoplasmic contents.

In wheat, oat, rye and barley, pollen structure development and viability are highly affected by boron deficiency. In rye, fertile pollen has higher boron content than sterile pollen. This indicated the higher boron requirement for the fertile pollen (Kaul, 1988).

The copper deficient plants develop minute anthers having scanty, dented, small, starchless abortive pollen (Dell, 1981). In wheat, oat and barley normal reproductive development, including microsporogenesis, is impaired due to copper deficiency. Wu and Li (1993) reported that the content of soluble calcium in the leaves and young panicles of Nongken 58S was significantly correlated with fertility transformation.

Potassium antimonate was used to locate calcium in fertile and sterile anthers of PGMS rice. During the development of fertile anthers abundant calcium precipitates accumulated in the anther walls and on the surface of pollen grains. In sterile anthers, calcium precipitates were less in anther walls and pollen grain surfaces and this anomalies in the distribution of calcium accumulation correlated with the failure of pollen development and pollen abortion (Tian *et al.*, 1998).

3.4.3.2. Biochemical changes during fertility alteration

2.4.3.2.1. Amino acids and proteins

Amino acids, the initial products of nitrogen assimilation, are the building blocks of proteins. Characteristic differences in bound and free amino acids have been detected between male sterile and male fertile plants. A reduction in the amounts of leucine, isoleucine, phenylalanine and valine occurred in the male sterile plants of barley and rice (Kern and Atkins, 1972). An increase in asparagine and glutamic acid was observed in wheat male sterile lines (Rai and Stoskopf, 1974). Alanine, glycine, arginine and aspartic acid were increased in male sterile lines of wheat, rice, sorghum and cotton respectively (Kaul, 1988).

Unlike in male fertile anthers, in male sterile anthers the proteins become hydrolysed to short chain peptides indicating the abnormal proteolysis in barley male sterile lines (Ahokas, 1980a). Because of alteration in amino acids content different or defective proteins are formed and a decreased or disturbed protein metabolism occurred in the male sterile lines of rice (Kaul, 1988).

Xiao *et al.* (1993) reported that the contents of free proline in the fertile anthers were higher than that of the sterile anthers and abnormal metabolism of free proline in sterile anthers was one of the causes for pollen abortion. But Elsy (1997) has contradicted the earlier reports and found that proline was accumulated more under sterile condition than in fertile leaves in TGMS 15, TGMS 16 and TGMS 18. Roystephen and Thangaraj (2000) reported the same results in TGMS 6 and TMGS 16. They also reported that the reduction of free amino acid content in TGMS lines.

Proteins being the immediate product of gene expression following transcription and translation, give an idea of the overall metabolic change under sterile or fertile conditions. The content of soluble protein in fertile anthers decreased from the differentiating stage of PMC to the late uninucleate stage and increased markedly after the binucleate stage (Peng and Wang, 1991). In sterile anthers, protein content decreased almost continuously. The total content of free amino acids was consistent with protein content. Thus it was suggested that protein synthesis inhibition in anthers was a basic cause of male sterility. Wang *et al.* (1991b) studied the variation in protein content of Nongken 58S at young panicle development stage by D, L-¹⁴C leucine labelling and two dimensional electrophoresis. The plants were treated for 10 h at 34.6°C or 14 h at 33°C with 28°C at night. About 200 protein bands with iso electric focusing (PI) of 4.1-7.2 and molecular weight of 10-95 kDa were obtained. Four bands varied significantly in intensity with photoperiod. Another four bands were present only in plants with long photoperiods

(sterile) and another band only in short day treated plant. It was concluded that the changes in fertility observed in the PGMS line was related to the variation of these proteins.

In a study on the changes in free and protein amino acids in leaves of PGMS lines Wang *et al.* (1993) found that during the photoperiod sensitive stage (II-V) of panicle differentiation, the contents of protein amino acids in the leaves of Nongken 58S decreased slightly, wherein, each amino acid and total free amino acids decreased gradually. As compared with SD treatments, LD slightly increased the contents of protein amino acids. Huang *et al.* (1994) reported that the soluble protein content of fertile anthers was higher than that of male sterile lines of PGMS. SDS-PAGE analysis showed differences in protein components with two more bands at 43 and 40 kDa in fertile anthers than the protein of male sterile anthers.

Wang and Weicheng (1996) reported the reduction in leaf soluble protein content of TGMS line Pei ai 64S during high temperature periods. Huang *et al.* (1996) also reported that the soluble protein content of anthers at 30°C was reduced in TGMS lines Erjiuqing S and Annang S and all pollen grains aborted compared with 25°C. SDS-PAGE showed bands with 13, 13.5, 23.5 kDa being absent in Erjiuqing S compared to its source line. At 30°C, 12 kDa band was absent compared to 25°C. Liu *et al.* (1995) extracted leaf protein from three male sterile lines at different growth stages under LD and SD and were separated using SDS-PAGE. They observed differences in the number and molecular weights of protein separated. At the intermediate stage of the glume, in male sterile cultivars, a protein band designated as PPOI was present in LD but not in SD. The band was present in LD and SD in normal cultivar.

Changes in soluble proteins of genetically male sterile *indica* rice varieties GD1S and N 19S were investigated under long day high temperature and short day low temperature regions and compared with normal variety Genghao 89. Only in the stages of pollen development, the lines had differences in soluble protein bands at positions 1, 2, 5, 9 and 10. During fertility conversion, the proteins expressed were rarely expressed during pollen abortion (Limei Ru *et al.*, 1999). Roystephen and Thangaraj (2000) observed a significant reduction of soluble protein content and qualitative and quantitative differences in leaf and anther protein profiles of fertile and sterile TGMS lines.

2.4.3.2.2. Carbohydrate fractions

Carbohydrate metabolism, being one of the most important metabolic pathways, will be the best choice for analysing any change in gene expression. The starch to sugar interconversion and the sugar transport are one of the basic factors deciding the development of panicle.

Once amino acids are altered, a disturbed carbohydrate metabolism occurs in the male sterile lines of rice (Kaul, 1988). During pollen maturation stage, male sterile anthers in wheat are deficient in sugars whose addition to the culture medium in which the male sterile wheat plants were grown with improved microsporogenesis (Ahokas, 1980a). A reduction in soluble sugar content of anthers and spikelets in Nongken 58S was reported by Peng and Wang (1991) under fertile condition. In sterile anthers only slight decrease in soluble sugar was noticed. Thus it was inferred that inhibition of starch synthesis was a basic cause of male sterility in Nongken 58S. Wang *et al.* (1991a) reported that the contents of starch, sucrose and reducing sugars in leaves in both long and short days increased from differentiation of the primary branch primordia until differentiation of pistil and stamens and then decreased gradually in Nongken 58. After stamen and pistil differentiation in the male sterile line, the sucrose content reduced but starch and reducing



sugars continued to increase slowly in short days and all the three carbohydrates in long days in male sterile Nonken 58S. Thus it was suggested that lack of transport of carbohydrates to the stamen might account for the male sterility. The content of reduced sugar at every development stage of young panicle in the leaves under sterility (LD) was more than that under fertility (SD) in Nongken 58S (He *et al.*, 1993).

Roystephen and Thangaraj (2000) reported that the carbohydrate fractions such as reducing sugars, total carbohydrates and total sugars were constantly increased from 25 DBH to 5 DBH in leaves of fertile TGMS lines and reduced in the same periods in sterile TGMS lines except starch content which showed constant increase in sterile lines compared to fertile ones. Similar results were also reported by Lakshmi Praba and Thangaraj (2000) in TGMS lines.

2.4.3.2.3. Nucleic acids

Impaired transcription and post transcriptional modifications are responsible for the specific changes in protein and enzymes. Many scientists have tried to study the change in ribonucleic acids, where the alteration would be mainly in mRNA level under sterility. In rice sterile mutants, the total nucleic acid content in male sterile anthers at the onset of meiosis was lowered by 54.63 per cent of the male fertile anthers (Kaul, 1988).

Yang and Zhu (1990) reported that the amount of total RNA from long photoperiod treatment (14 h) was 6.3-100 per cent lower than that from SD treatments in PGMS lines. They observed a small molecular weight RNA fraction in LD at fertility transformation stage and suggested that this RNA fraction may be associated with fertility transformation. The DNA and RNA contents from the fifth leaf to seventh leaf of Nongken 58S and Nongken 58 both under LD and SD inductions declined gradually and

equally, while the RNA content in the leaves above the seventh leaf segment increased differentially (Lu *et al.*, 1993)

Changes in total RNA and DNA contents were studied in TGMS *indica* rice varieties and results showed that the total RNA and DNA contents of sterile lines were much lower than the fertile ones and the normal variety Zike at PMC and PMC meiosis stages (Shu and Chen, 1999). Roystephen and Thangaraj (2000) studied the RNA content of fertile and sterile TGMS lines TGMS 16, TGMS 18 and TGMS 29 and reported that there was a constant increase in RNA content in fertile leaves from panicle initiation to flowering stage while in sterile lines RNA content was reduced constantly

2.4.3.2.4. Enzymes

High enzyme activity occurs in the areas of high metabolic activity, rapid cellular differentiation, protoplasmic synthesis and vascularization. Shen and Cao (1992) studied the alteration of some enzyme activities in PGMS line Nongken 58S and reported that the activity of NRase was decreased under long day length with increased temperature condition and the activity of superoxide dismutase also decreased during sterile condition compared to fertile condition. During the development of anthers, the peroxidase activity of the sterile anthers had a change from high to low level and the reverse for fertile anthers (Cheng and Xiao, 1993). The changes in the activities of IAA oxidase, peroxidase (POX) and superoxide dismutase (SOD) were closely related with the transformation of fertility in Nongken 58S (He and Xiao, 1993). The activity of POX in young panicles in fertile condition was higher than under sterile condition especially before the period of meiotic divisions of PMCs.

Zang *et al.* (1994b) reported that the activities of POX, CAT and SOD were higher in fertile phase than in panicles and anthers of PTGMS rice from sterility to fertility

transformation. Ascorbate peroxidase, glutathione reductase and glucose-6 phosphate dehydrogenase activity increased in the fertile anthers with maximum activity at the uninucleate stage. In male sterile anthers, the activity gradually decreased from the early uninucleate to the trinucleate stage. Compared to fertile anthers, there was 6, 19 and 22 per cent activity respectively in male sterile anthers. There was a decrease in malic enzyme, malate dehydrogenase (MDH) and isocitrate dehydrogenase activity in male sterile anthers. The reduction potential of male sterile anthers was lower than in fertile ones indicating that the low reduction potential might have caused uncontrolled activated oxygen molecules and hence anther sterility (Liang *et al.*, 1995).

Zhu and Cao (1997) studied the POX activity in fertile and sterile anthers of *indica* and Japonica rice hybrids and reported that the POX activity was higher in the fertile anthers than in the sterile anthers of hybrids. Wang and Weicheng (1996) reported that catalase and leaf acid phosphatase activities were decreased during sterile condition compared to fertile condition in TGMS line 'Pei ai 64S. Chen and Zhou (1997) studied the activity of certain enzymes in leaves of photo and thermosensitive genic male sterile lines and revealed that the activities of RNase and cytochrome oxidase were increased and acid phosphatase, peroxidase activities were decreased during high temperature with long days. Li Peng *et al.* (1997) investigated the changes in NAD^+ malate dehydrogenase (MDH) and acid phosphatase (AP) activities in TGMS line Pei ai 64S during PMC formation, reduction division and pollen ripening and reported that the abortion of pollen grains was related to the decrease in MDH activity in spikelets and also closely related to acid phosphatase activity. Roystphen and Thangaraj (2000) reported that the free radical scavenging enzymes *viz.*, superoxide dismutase, peroxidase and catalase showed reduced activity in sterile TGMS lines. Roystphen (1998) reported that the activity of enzymes IAA oxidase and ascorbic acid oxidase was higher under sterile condition in TGMS lines.

2.4.3.2.5. Isozymes

Li *et al.*, (1994) reported that isozymes play an important role on regulating plant growth and development. Hu and Wan (1991) studied the correlations between differences in isozyme patterns and male fertility under different natural conditions, day length and temperature regimes by means of pollen fertility index using PTGMS line Nongken 58S, Shuang 8-S and N98S. Tong *et al.*, (1993) studied the linkage relationships between isozyme genes and male sterility genes. They observed a close relationship between Adh-1, Est-3 and male sterility. One of the sterility genes were linked Adh-1 with a recombination value of zero and another gene was linked to Cat-1 with recombination value of 29 per cent. Long days (sterility) did not change the number of isozyme bands of four enzymes analysed but decreased their activity. But Liu *et al.*, (1993a) observed differences in banding patterns of peroxidase isozyme in Nongken 58S in SD and LD at stages II and IV. At stage II, LD treated plants gave two extra weak bands compared to SD treatment, where as at stage IV, one weak band was present in SD treatment and absent in long days. At stages V and VI, they got similar patterns for LD and SD treated plants. Liu *et al.*, (1993a) studied the variation of POX isozyme and glutamic-oxaloacetic transaminase (GOT) isozyme in leaves of Nongken 58S under different photoperiodic conditions and reported significant differences in isozyme band number and intensity of POX and GOT. The findings suggested that the changes of enzyme activity and variation of isozyme electrophorogram in NK 58S would relate with the alterations of some metabolic pathways under LD. The variation in peroxidase isozymes and its activities in leaves of 105S and 105 as in the case of Nongken 58S and Nongken 58 also studied by Wu and Xiao (1993)

Li *et al.*, (1994) observed a specific esterase band (S band) in Annong 1S and N42S (a PGMS line from Nongken 58S). Annong 1S and 210 and other normal varieties did not have the 'S' band. They reported stable expression in mature leaves and

unstable expression in young leaf blades. Li *et al.*, (1995) detected Est-1 and Est-2 isozyme bands at all growth stages of Annong 1S and Est-3 band only at mature leaf stage. Li Peng *et al.*, (1997) studied the banding pattern of acid phosphatase and MDH isozymes in TGMS line Pei ai 64S and they reported significant differences in isozyme band number and intensity of bands between fertile and sterile conditions. Isozyme analysis of peroxidase, esterase and superoxide dismutase enzymes showed polymorphism with distinct isoform bands in fertile and sterile leaves of TGMS lines TGMS 16 and TGMS18 (Roystephen, 1998). Lakshmi Praba and Thangaraj (2000) reported that the difference in isozyme banding pattern of peroxidase under fertile and sterile conditions could be used as a biochemical marker for identifying TGMS character in rice.

2.4.3.2.6. Molecular markers

Rice has a relatively small genome and well developed linkage maps. It is possible to map the TGMS and PGMS genes using molecular markers. Once the genes are mapped, it can be cloned and transferred to other crops, which will greatly advance the practical application of hybrid production to the entire field of crop breeding.

The TGMS gene of 5460S was mapped with RAPD and RFLP markers, using bulked segregant analysis (Wang *et al.*, 1995b), later they screened 400 RAPD markers for polymorphism between parents and F₂, representing fertile and sterile plants. They reported that only four primers produced polymorphic products and only one single copy fragment found (a 1.2 kb fragment) and amplified by primer OPB-19 and named as TGMS 1.2 mapped on chromosome 8 and located about 6.7 cM from the TGMS gene. Wang *et al.*, (1995c) have reported 5 polymorphic products with 2 single copy fragments amplified by OPB-19, and named as TGMS 0.5 and TGMS 1.2 respectively. Rice BAC (bacterial artificial chromosome) library was screened with ³²P labelled TGMS 1.2 as

probe. The average insert size of the positive clones was about 150 kb and the largest insert was 240 kb.

Subudhi *et al.* (1995) found four polymorphic random primers *viz.*, F18, AA7, AC1 and AC3 to be linked to the TGMS gene. Wang *et al.* (1995b) detected polymorphism with RAPD marker OPAD-19, which amplified a 2.3 kb fragment, which was absent in sterile parent and sterile F2 individuals. Subudhi *et al.* (1997) used F2 population from IR 32364 x IR 68 to map TGMS gene tms 3(t). Four RAPD markers were identified in which three, OPF 182600, OPB 19750 and OPAA 7550 were linked to tms 3(t) in repulsion phase. One OPAC 3640 was linked to tms 3(t) in coupling phase. Dong *et al.*, (2000) studied the F2 population developed from a cross between a TGMS *indica* mutant, TGMS-VN1 and a fertile *indica* line CH1 to identify molecular markers linked to the TGMS gene. They performed bulked segregant analysis using AFLP technique and four AFLP markers linked to the TGMS gene. All the markers were linked to the gene in coupling phase. Reddy *et al.* (2000) reported that the microsatellite RM 257 located on chromosome 9 was linked with TGMS trait in SA2 at a distance of 6.2cM. RM257 produced a codominant polymorphism with 145-6P(sterile) and 132-6P (fertile) products. They suggested that both individually and collectively, the markers TS2000 and RM257 located on either side of the TGMS locus are very useful for marker-aided selection.

2.5. Morphological and anatomical changes associated with male Sterility in EGMS lines

In flowering plants, male sterility is due to abnormal development of the male gametophyte resulting in pollen abortion (Kaul, 1988). Chen *et al.* (1990) investigated the anther and pollen of Annong 1S, a photoperiod and temperature sensitive genic male sterile rice by scanning electron microscope. They observed that under long day length and

high temperature anthers were slender, did not dehisce and had 100 per cent pollen sterility and the pollen development having stopped at the monokaryon stage.

Observations on the changes in anther microstructure of PGMS rice in LD and SD conditions showed that (1) there were vessel elements in sieve tubes in vascular bundles of anther septa in SD but not in long day treated plants (2) vessel elements, sieve tubes and parenchyma cells developed poorly and sheath cells wrinkled or out of shape and (3) in contrast to the SD treated plants anther cytoplasm of tapetal cells did not degenerate in LD treated plants. It was suggested that the abnormal alterations in the vascular bundle and tapetum tissue may reduce nutrient supply to developing microspores in LD treated anthers and this may be one of the reasons for sterility (Wang *et al.*, 1992).

Lu *et al.* (1993) studied the ultrastructural changes during microsporogenesis and pollen development in Nongken 58S by transmission electron microscopy. Ultrastructural abnormalities like aggregation of ribosomes, gradual disintegration of endoplasmic reticulum, plastids, mitochondria and other organelles, absence of starch accumulation, increase of phagocytic vacuoles, thinning of the cytoplasm and ultimately abortion of pollen grains were observed under long photoperiodic treatment (>14 h).

The abortive process of pollen of five genetically male sterile (GMS) rice lines was studied by Wu *et al.* (1994). The membrane between two daughter nuclei in the first meiotic division failed to form. The CGMS line Zhenshan 97 A had typical nuclear degenerative pollen abortion while all the GMS lines were nuclear proliferative types, it was suggested that this type of ms was very stable producing flowers with dehiscent anthers.

Sun and Zhu (1995) studied the ultrastructure of pollen and anther wall development in Hubei PGMS line Nongken 58S. It was observed that under long day, the organelles degenerated dramatically and pollen grains were irregular in shape and pollen wall was abnormally developed. The tapetum degenerated by the microspore stage in fertile anthers but in sterile anthers it was not degenerated and the degeneration of the middle layer of the sterile anther was also delayed. Prasad and Rao (1996) studied the male sterility in different GMS lines of rice. There were differences in the thickness of anther sac layers. ie. inner tapetum, middle endothecium and outer epidermis. The reason for pollen abortion may be due to incomparability between nuclear and cytoplasmic genes. The results of Feng *et al.* (2000) showed the abnormalities of microsporocytic cytoplasm or pollen exine rather than the tapetal development caused pollen abortion in TGMS line Pei ai 64s. Lakshmi Praba (1998) reported that the microtomy sections of male sterile anthers showed persistent tapetum whereas in male fertile anthers tapetum degenerated with thickened endothecium and epidermal cells.

2.6. Induction of male sterility by chemicals and plant growth regulators

Chemicals capable of selectively inhibiting pollen development and thus blocking male fertility have been known for some time. Most were identified in screening programmes designed to discover chemical hybridizing agents (CHAs) for the large scale commercial production of hybrid seed, particularly for small grains. Research into the mode of action of CHAs has lagged behind their practical applications. The following four classes of CHAs as far as known are.

1. Plant growth regulators and substances, which disrupt floral development eg. Plant hormones, such as auxins, gibberellins and abscisic acid. Synthetic growth regulators such as LY 195259 and TD 1123.
2. Metabolic inhibitors such as arsenates

3. Inhibitors of microspore development such as copper chelators, ethylene, fenridazon, phenylcinnoline carboxylates and genesisR.
4. Inhibitors of pollen fertility such as acetidine-3-carboxylate. (Cross and Schulz, 1997).

Two line breeding involving chemical emasculation is regarded as an alternative to male sterile maintainer-restorer system. The functional male sterility in the female parent is obtained with a chemical rather than by genetic manipulation. China is probably the only country where CHAs are being used in commercial hybrid seed production.

Efficiency of chemicals in hybridizing work depends on the criteria like, there should be a source of viable pollen from a male parent that can out cross, synchrony in flowering for both parents, abundance of pollen from male parent and good flower opening characteristics in the female parent. Obtaining male sterility by a simple chemical treatment without an accompanying adverse effect on female sterility have many advantages in two line method like a wide range of varieties can be used for making superior hybrid combinations. Instability of male fertility expression produces selfed seeds among hybrid seeds and reduces the quality and value. Such partial male sterile lines can be made completely sterile with a spray of potential chemical (Ali, 1996). An ideal chemical should have the qualities like, wide action spectrum to induce sterility in successively emerging panicles, selective and total sterilization of stamens without affecting ovular fertility, least phytotoxic and non-carcinogenic with no residual toxicity which is harmful to human beings and animals, easy to apply and economical (Ali, 1995a).

When sprayed on rice plants at specific developmental stages, a gametocide or chemical hybridizing agent (CHA) can emasculate the plants. Thus resulting in male sterility while maintaining normal female fertility (Lasa and Bosemark 1993).

Many scientists have reported that ethephon induces male sterility of crops (Bennett and Hughes 1972; Perez *et al.* 1973; Hughes 1976; Parmar *et al.* 1979; Chan and Cheah 1983). More than 50 chemical emasculators have been identified for more than 40 crops, but most also damage the pistil or cause abnormal flowering (Wang *et al.* 1981, Yu *et al.* 1991). For rice, male sterility is induced by using 0.4 per cent EMS for 48 h at 10°C (Minocha and Gupta 1988; Minocha *et al.* 1991). Aswathanarayana and Mahadevappa (1991) reported that 800 ppm of GA, 8000 ppm of ethephon, 0.02 per cent maleic hydrazide (MH), and 0.8 per cent 2, 4-D induced a high level of male sterility in rice. Kitaoka *et al.* (1991) reported that the male sterility reached 95 per cent or more using isourea at 3 kg ha⁻¹ + ethephon at 5000 ppm or by alternatively using isourea at 10 kg ha⁻¹ + ethephon at 25000 ppm. In the 1970s and 1980s, China developed chemical emasculators using arsenates, such as male gametocide 1 (Zinc methyl arsenate) and male gametocide 2 (sodium methyl arsenate) while they caused excellent emasculation of rice, they were very toxic to the environment.

In experiments with cucurbits, Wittwer and Hillyer (1954) could suppress the male flowers with maleic hydrazide, while the female flowers seemed normal and fertile. Kho and De Bruyn (1962) found that dichloro acetate had a positive action on the pollen sterility of *Antirrhinum*. Complete male sterility was induced by a spray of a solution (0.5%) applied before two weeks of flowering. Rehm (1962) stated that 25-50 ppm triiodo benzoic acid induced male sterility in tomato for 8 weeks without damage to female fertility, while maleic hydraxide 500 ppm acted on the pollen of the same crop and cause male sterility for two weeks. Sharma (1979) reported that the spray of FW 450 (α , β dichloro isobutyrate) solution on pearl millet induced functional male sterility by checking the dehiscence of anthers. Dalapon has been reported to have a similar effect on cotton (Scott, 1961). Vander veen and Wirtz (1968) reported that after EMS treatment of

Arabidopsis seeds it is easy to select lines segregating genic male steriles with complete expression of male sterility with unreduced female fertility.

Male sterile flowers were obtained in all GA₃ treated lettuce plants regardless of GA₃ concentration and frequency of spraying. Some of the male sterile flowers had florets with shrivelled anthers, which released no pollen, others had no anthers at all. All florets seemed to have normal pistils (Eenink, 1977).

The use of ethephon (2-chloroethyl phosphonic acid) as a possible gametocide was based on the initial observation that foliar applied liquid ethephon increased the number of pistillate flowers on monoecious cucumbers (McMurray and Miller, 1969; Robinson *et al.*, 1969). Since then liquid ethephon has been used experimentally as gametocide on both wheat and barley (Brown and Earley, 1973; Stoskopf and Law, 1972) and levels of male sterility upto 100 per cent have been obtained with no apparent reduction in female fertility (Fairey and Stoskopf, 1975).

In winter rye 2-3-dichloro isobutyrate application has been shown to affect the development of pollen (Aung 1977). Odeigah *et al.* (1996) studied the effect of EMS and sodium azide on male sterility induction and reported 100 per cent induced sterility in cowpea. Kumar and Singh (1983) reported the positive effect of EMS on barley in inducing male sterility without affecting female fertility and recommended for large scale hybrid seed production in highly self pollinated crops. Glenn and Wayne (1976) reported the induction of male sterility by seed treatment of ethidium bromide at 1000 ppm in pearl millet.

The application of GA₃ at 90-100 ppm on the 3rd day after flower bud initiation induced a high degree of male sterility without affecting female fertility in sunflower

(Seetharam and KusumaKumari, 1975). Ladislav and Marie (1978) obtained more than 90 per cent of male sterility in wheat by spraying of ethrel 1500 ppm at the booting stage of the last leaf and also reported that the female fertility was not affected and cross pollination was possible.

Two chemosterilants, RH 531 and RH 532, were developed by Rohm and Haas to use primarily on cereals. They induced complete sterility in barley and wheat when applied as a foliar spray (Rajendra and Bates, 1981). Thakur and Rao (1988) reported that foliar spray of 2000 ppm ethrel on pearl millet at lateboot stage increased the male sterility than at early boot stage and ethrel spray was not affected the female fertility of pearl millet.

The changes in ethylene biosynthesis in young panicles of PGMR (Photoperiod-sensitive genic male sterile rice) cv. Nongken 58S and its normal parent line cv. Nongken 58 was studied and the results showed that ethylene biosynthesis in young panicles of NK 58S was regulated jointly by photoperiod and temperature. A significant negative correlation was found between ethylene release rate (ERR) of young panicles of NK 58S and corresponding pollen fertility. When plants were treated with AVG (amino ethoxyvinyl glycine) to retard ethylene biosynthesis the sterility of NK 58S was obviously reversed. When plants treated with ACC to stimulate ethylene biosynthesis, the fertility level of NK 58S decreased sharply. It is concluded that ethylene biosynthesis is closely correlated with the fertility expression of PGMR and ethylene plays a major role in the regulation of fertility alteration of NK 58S (Li DeHong *et al.*, 1996).

Pandey *et al.* (1996) reported that the treatment of streptomycin sulphate and sodium azide at 0.5 per cent and 0.025 per cent respectively caused white and empty anthers in pigeon pea.

Huberman *et al.* (1997) reported in pepper that high temperatures increased ethylene production in reproductive organs and also reduced the IAA levels and particularly auxin transport capacity in reproductive organs. This was the major mechanism by which high temperatures caused reproductive organs abnormal.

The pyridine derivative hybridizing agent TO_3 applied at 0.03 g m^{-2} at 12-21 days before panicle emergence induced 97.2 per cent spikelet sterility. Sterility was 66.3-89.31 with application at 22-28 days before panicle emergence and 84.2-93.0 per cent with application 5-11 days before panicle emergence. The main effect of TO_3 was reduction in number of stamens and they became small and producing few pollen grains in rice (Zhang *et al.*, 1995).

Tian *et al.* (1999) reported the reduction in pollen fertility by the application of ethrel or ACC in CMS rice and it can be reversed by the application of AVG by changing the metabolism of protein, DNA and RNA.

Ali *et al.* (1999) studied the male sterilizing efficacy of 29 oxinilate formulations in *indica* rice cv Pusa 150 and reported that ethyl 4' fluoro oxinilate and ethyl 4' bromo oxinilate were the most potent, inducing the highest pollen sterility with least phytotoxicity.

Shamsai *et al.* (1996) tested three chemical gametocides on four genotypes of rice. Among the chemicals, ethrel and sodium methyl arsenate were found to induce the highest rates of pollen sterility in all genotypes of rice. The chemical hybridizing agents TO_3 (Pyridone derivative), I_1 , I_2 and I_3 (Pyridoxinone derivatives) were tested on rice. I_2 and I_3 induced complete male sterility in cv Guangluai 4 applied 15 days before heading (Zhang *et al.*, 1996).

Singh (1999) tested four aqueous concentrations of Nirma detergent (3, 4, 5 and 6%) for inducing male sterility on rice plants of cv AKS1 at tillering and initial booting stage. The lowest concentration (3%) of the detergent induced the highest level of male sterility (93.3%) at tillering. At initial booting stage, highest levels of male sterility were noted for the highest detergent concentration (6%).

Manjula and Ibrahim (1999) tested the effect of ethrel, MH and streptomycin on pollen sterility in rice and the results revealed that MH only induced high pollen sterility (>90%) and caused anther deformation and thus high spikelet sterility.

Tien ChangEn (2000) reported that exogenous application of ethrel decreased the frequency of fertile pollen grains in the maintainer line Zhenshan 97B and AVG application reduced the ACC content and ERR of both CMS and maintainer line and partially restored the pollen fertility in the CMS line. Exogenous ethrel however counteracted the effect of AVG and excess release of ethylene might be a cause of CMS in rice.

In maize x sorghum cross, pollen germination and entry of pollen tube in maize silk were normal but did not reach embryo sac to allow fertilization. To overcome this barrier, treatment with chemicals 2, 4-D, GA₃ and salicylic acid (SA) was conducted either one day before or one day after pollination. The growth hormones 2,4-D and GA₃ had favourable effects on pollen tube growth whereas the SA, an immuno suppressant, had inhibitory effect (Manickam and Sarkar, 1999). Thangaraj and Lakshmi Praba (2000) reported that foliar spray of salicylic acid at 800 ppm during panicle initiation stage induced 100 per cent pollen sterility in TGMS line 29.

2.6.1. Biochemical changes during fertility alteration by chemicals and plant growth regulators

Treatment of chemical hybridizing agent "Bao Chungling" (BCL) caused several physio-biochemical changes in the TPGMSR lines Henghong S1 and Pei ai 64S. These included increase in acid soluble inorganic phosphorus in spikelets and decrease in soluble carbohydrates, RNA and DNA contents in leaves and spikelets of both cultivars (Liu *et al.*, 1998).

Tian *et al.* (1999) studied the effect of ethylene on metabolism of protein, RNA, DNA and activated oxygen, catalase, SOD and peroxidase in the leaves and panicles of male sterile rice and reported that the treatment with ethrel or ACC markedly reduced these biochemical parameters and it can be reversed by the application of AVG.

Ethylene release rate (ERR) was more under sterile condition in TGMS lines and the application of AVG reduced the ERR and reduced the sterility percentage of TGMS lines. AVG application also promoted the polyamine content which increased the pollen fertility in TGMS lines (Tian ChangEn *et al.*, 1999). Exogenous ethrel decreased the percentage of fertile pollen in the CMS line Zhenshasan 97, and exogenous AVG reduced the ACC content and ERR and restored partially the pollen fertility in the CMS line. However exogenous ethrel counteracted the effect of AVG (Liang *et al.*, 1999).

Chenzhi *et al.* (1997) reported that salicylic acid (SA) sensitive catalases serve as biological targets of SA in rice tissues and also the salicylic acid insensitive catalases were inhibited by SA application. Tang *et al.* (1996) studied the relationship between induced male sterility and endogenous hormone levels and reported that the male sterility induced by TO_3 caused reduction in IAA and GA_3 levels and increase in ABA levels in young panicles and anthers of treated rice plants.

Application of I₂ and I₃ (pyridoxinone derivatives) decreased the total nucleic acids, RNA in anthers, protein and proline contents in rice variety Guangluai 4 and it was concluded that male sterility induced by I₂ and I₃ is closely related to deficiencies in nucleic acids, protein and proline during anther development (Zhang *et al.*, 1996).

Limei Ru *et al.* (1999) reported that during induction of sterility by the spray of ethrel, the soluble protein content was reduced in dual purpose genic male sterile lines GD1S and N19S. Experiment with foliar spray of salicylic acid (50-1000 ppm) on physiological responses of cotton revealed that salicylic acid spray generally decreased the total soluble sugars, although effects varied with time and application rate (Shehata *et al.*, 2000).

Xiao *et al.* (1996) reported that the CHA "Jinaolin" (0.5 kg ha⁻¹) at the immature ear stage in wheat caused 98.28 per cent pollen sterility and reduction in carbohydrate content and increase in proline accumulation significantly.

Barley plants treated with salicylic acid (SA) @ 100 µ mol l⁻¹ to 1 m mol l⁻¹ showed a decrease in levels of total soluble protein, in particular in the level of Rubisco (Pancheva and Popova, 1998).

Spraying mustard plants with salicylic acid (10 to 500 µM) significantly reduced both H₂O₂ and catalase activity below control levels (Dat *et al.*, 1998).

Potato microplants propagated as nodal explants were subjected to acetyl salicylic acid (ASA) treatment and the treated plants showed significantly less catalase activity and higher level of H₂O₂ than controls (Lopez *et al.*, 1998).

Young maize plants treated with 0.5 mM salicylic acid showed significant decrease in net photosynthesis, stomatal conductance, transpiration and intercellular CO₂ concentration at normal growth temperature (Janda *et al.*, 1998). It was concluded that the exogenous application of salicylic acid decreased the photosynthetic capacity of maize plants. Soaking of maize seeds in salicylic acid solution (0.1%) caused significant reduction in protein N, NRase activity in Maize (Asthana and Srivastava, 1977).

Thangaraj and Lakshmi Praba (2000) reported that salicylic acid spray at 800 ppm during panicle initiation stage caused pollen sterility upto 100 per cent. This induced male sterility was found to be associated with an increased activity of enzyme IAA oxidase and accumulation of phenols, which cause thermogenesis in TGMS lines, would be a possible reason for pollen sterility.

Materials and methods

CHAPTER III

MATERIALS AND METHODS

The present investigation was carried out primarily to understand the physiological, biochemical and molecular mechanisms of thermosensitive genic male sterility (TGMS) in rice. An attempt was also made to manipulate the fertility / sterility in TGMS lines with chemicals and plant growth regulators. This research work was carried out in the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, during 1998-2001. A brief account of the materials used and methodologies followed in different experiments in the present study are given below.

3.1. Experiment I

Identifying critical sterility temperature

Three TGMS lines, which were reported to have stable sterility and supplied by the Centre for plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore formed the basic material for the study. The details of these lines are given in Table 2.

The TGMS lines were sown sequentially once in 20 days in plastic pots of 20 cm diameter keeping two plants per pot in the glass house of the Department of Crop Physiology. Properties of the soil used for pot culture experiment are given in Table 3.

Four replications were maintained for each line in each set. After studying the various developmental stages and growth duration of the TGMS lines, sowing date was adjusted so that the lines had similar reproductive stage. Package of practices as recommended in crop production guide of TNAU for rice crop was followed for maintenance of the crop.

Table 2. Details of TGMS lines used for studying the critical sterility temperature

Entry	IRRI designation	Parentage
TGMS 6	RP 2161-106-1-1	-
TGMS 16	IR 68945-44-33-4-14	Norin PL 12/IR 36
TGMS 29	CTGMS	-

Table 3. Properties of soil used for pot culture experiment

Properties of soil	
Mechanical composition	
Course sand (%)	15.4
Fine sand (%)	34.6
Silt (%)	16.2
Clay (%)	33.8
Texture	Clay loam
Physio chemical properties	
Silt reaction (pH)	7.9
EC (dSm ⁻¹)	0.35
CEC (me 100 g ⁻¹)	38.5
Nutrient status	
Available N (kg ha ⁻¹)	252.0
Available P ₂ O ₅ (kg ha ⁻¹)	10.8
Available K ₂ O (kg ha ⁻¹)	536.7

The mini phytotron (Conversion E15-Convion products company, Manitoba, Canada) available in the Department of Crop Physiology was used in this experiment. The temperature regimes at which the growth chambers were maintained to get the required daily mean temperature are given in Table 4.

3.1.1. Description of the Phytotron

The internal dimension of the growth chamber (inside space) was 6.0+2.5+1.0 ft (lxbxh). Sixteen fluorescent tubes and 16 incandescent bulbs were fitted on the top of the chamber to get similarity in spectral characteristics of natural PAR. The height of the chamber was adjustable so as to increase or decrease the light intensity requirement at the canopy level. The temperature can be set between 10°C to 50°C with an accuracy of 1°C. The light intensity can be varied from 0 to 800 μ moles $m^{-2} sec^{-1}$ in maximum height position which was half the natural light intensity at 12.00 noon. Relative humidity can be adjusted from 20 to 95 per cent. For humidity maintenance, distilled and demineralized water was supplied to avoid any salt deposition inside. For getting a constant current the phytotron was connected with 10 KVa voltage stabilizer. The parameters *viz.*, light intensity, temperature, relative humidity at the required levels were fed to the instrument with a set clock adjustment and the temperature of the chamber was recorded with a recording device. Any shift of temperature from the set temperature was indicated with an alarm facility.

The light duration of the chamber was adjusted to short day condition (10 h / day) to avoid any possible influence of long days in inducing pollen sterility. Light intensity of 400-800 μ moles $m^{-2} sec^{-1}$ was set according to diurnal variation from morning to evening with relative humidity varying from 60 to 80 per cent. The potted plants were transferred to the phytotron at 25 days before flowering and kept upto 5 days before

Table 4. Thermoperiod conditions in phytotron

Time (hrs)	Treatment temperatures (°C)								
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉
06-10	34	33	32	32	32	31	30	30	28
10-16	36	35	34	34	34	33	32	32	30
16-20	32	31	30	30	30	29	28	28	26
20-06	24	23	23	22	21	21	21	20	20
Max (°C)	36	35	34	34	34	33	32	32	30
Min (°C)	24	23	23	22	21	21	21	20	20
Daily mean (weighted average)	30.0	29.0	28.5	28.0	27.5	27.0	26.5	26.0	25.0

Day length : 10.00 h day⁻¹ (08:00-18:00 h)

RH : 60-80 per cent

Light : 400 – 800 $\mu\text{moles m}^{-2} \text{s}^{-1}$

heading (20 days duration). Pollen sterility behaviour of these plants was observed at the time of flowering. Five florets were collected from each panicle, anthers of these florets were squashed, and the pollen grains observed under microscope after staining with I-KI 1 per cent solution. Mean values of the percentage pollen sterility (unstained pollen grains) from these microscopic fields were considered for determining the critical sterility temperature of the lines.

3.2. Experiment II

Identifying critical stage of thermosensitivity

The critical stage of thermosensitivity was the differentiation of secondary rachis branches to the formation of pollen mother cells (Yuan, 1990). Yao *et al.* (1995) found that it was 5 to 20 days before flowering. With this information in the present investigation, the TGMS lines were exposed to sterility inducing temperature with different duration during reproductive stage. The pollen sterility at flowering was noted, for identifying the critical stages of thermosensitivity. Stage of panicle development and corresponding days before heading was assessed following the criteria as given in Table 5.

The TGMS lines were grown in plastic pots and maintained in glass house during winter season till they were transferred to the phytotron. Four replications were maintained in each set for each lines. All the cultural operations were followed as described in crop production guide of TNAU for rice crop.

Table 5. Stages of panicle development in rice

Stages	Duration of stages (days)	Features	Days before heading
I	2	Differentiation of first bract primordium	30-32
II	3-4	Origination of primary branch primordium	27-30
III	4-5	Origination of secondary branch primordium, young panicle about 1mm long and covered with white hairs	22-26
IV	6-7	Appearance of glumes, differentiation of stamen and pistil, young panicle about 0.5-1.0 cm long	19-22
V	3	Formation of pollen mother cell, floret about 1-3 mm long, young panicle 1.5-5.0 cm long	16-19
VI	2	From prophase I of meiosis to formation of tetrad, floret about 3-5 mm long, young panicle about 5-10 cm long	12-15
VII	7-9	Filling phase of pollen, floret and panicle reach full length and colour turns to green	9-11
VIII	2	Matured pollen, panicles are going to emerge	2

(Rangaswamy, 1993)

Experimental details

Entries - TGMS 6, TGMS 16, TGMS 29

Treatments [days before heading (DBH)]

T ₁	- 0-30 DBH
T ₂	- 20-30 DBH
T ₃	- 5-25 DBH
T ₄	- 5-20 DBH
T ₅	- 5-15 DBH
T ₆	- 10-25 DBH
T ₇	- 10-20 DBH

Replication : Four

Design : CRD

The phytotron was set to an average temperature of 29°C. Mean of the pollen sterility data were used to identify the critical stage of thermosensitivity.

3.3. Experiment III

Physiological and biochemical changes during fertility alteration

The three TGMS lines along with a normal variety ADT 36 formed the basic material for this experiment. Plants were grown in the glass house in plastic pots. The plants were transferred to the growth chamber, when they were in stage III of panicle development (25 days before heading) (Plate 1) The temperature treatment given in the phytotron to imitate the diurnal variation of summer season for induction of male sterility is given below.

Time (h)	Temperature (°C)	RH (%)	Light intensity $\mu\text{M m}^{-2} \text{s}^{-1}$
08.00-10.00	34°C	80	600
10.00-12.00	35°C	70	800
12.00-16.00	36°C	60	800
16.00-18.00	35°C	60	600

3.3.1. Experimental details

Entries	- TGMS 6, TGMS 16, TGMS 29 and ADT 36
Treatment	-
	T ₁ - Fertile
	T ₂ - Sterile (phytotron)
Replication	- Five
Design	- FCRD

Sowing date was adjusted so that the lines came to panicle initiation stage during 1st week of December. The mean maximum and minimum temperature for December and January were 29.4 and 17.9°C. Five pots each with three plants were kept for each variety to have sufficient samples for analysis. Fertile set of TGMS lines with ADT 36 which was used to compare with TGMS lines, were kept in the glass house. The plants were kept inside the phytotron upto stage VII of panicle development. Leaf samples were collected at 5 day interval from 25 days before flowering to get the exact stage of fertility alteration in terms of physiological and biochemical changes and also young panicles were collected at 10 DBH and heading stage for biochemical analysis.

3.3.2. Physiological parameters

3.3.2.1. Gas exchange measurements

Gas exchange parameters *viz.*, photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-1} \text{ s}^{-2}$), transpiration rate ($\mu \text{ g H}_2\text{O m}^{-2} \text{ S}^{-1}$) and stomatal conductance (cm s^{-1}) were recorded using an advanced portable CO₂ gas analyzer (model CL-CID Inc, USA). The readings were taken during 11:00 to 12:00 noon on clear sunny day when the PAR was more than 1000 $\mu \text{ moles m}^{-2} \text{ s}^{-1}$. Third fully opened leaves from the top were clamped to the leaf chamber and held perpendicular to the incident light and only the computed values were taken. The instrument was working in open type principle thus maintaining a constant CO₂ flux to the leaf chamber which is at ambient concentration. RH was also maintained

at a steady state level equal to the ambient RH to simulate a condition very similar to that of the ambient air.

3.3.2.2. Hill reaction

Hill reaction was determined by recording the reduction of 2,6- Dichlorophenol indophenol (DCIP) as described by Fleischhacker and Senger (1978).

Isolation of chloroplast

One g of fresh leaf was cut into small bits and ground in a pre-chilled glass pestle and mortar using chilled isolation medium. The homogenate was filtered and centrifuged at 4000 rpm for 5 minutes. The supernatant was discarded. The pellet was resuspended with isolation medium and centrifuged at 7000 rpm for 5 min. The supernatant was discarded and the pellet was resuspended in 1 ml of resuspension buffer and stored in ice and used within two hours for estimation. The whole procedure was carried out at about 4°C temperature.

Reagents

A. Isolation medium	330 mM sorbitol
	5 mM Mg Cl ₂
	10 mM NaCl
	2 mM sodium ascorbate
	20 mM Tris-HCl (pH 6.5)
B. Resuspension medium	330 mM sorbitol
	2 mM EDTA
	5 mM MgCl ₂
	10 mM NaCl



C. Reaction mixture/buffer	20 mM Tris-HCl (pH 7.5)
	10 mM NaCl
	10 mM MgCl ₂
	100 mM sucrose
	150 mM NH ₄ Cl
D. Dye	20 mM DCIP

Estimation of Hill reaction

The cuvette containing 2.87 ml of reaction buffer, 0.02 ml of chloroplast suspension and 0.10 ml of NH₄Cl (150 mM) was placed in the spectrophotometer (Beckman DU-640) and the absorbance was adjusted to zero at 600 nm. The cuvette was taken out and 0.01 ml of 20 mM DCIP was added and the initial absorbance was measured. The cuvette was then taken out and illuminated with the light from a 200 watt bulb for 30 second and the final OD was measured immediately. The difference in absorbance was due to the reduction of DCIP, and therefore the difference in OD (delta OD) was used to calculate the amount of DCIP reduced. The extinction coefficient of DCIP was calculated by means of 2.89 ml reaction mixture, 0.10 ml NH₄Cl (150 mM) and 0.01 ml DCIP (20 mM).

The total chlorophyll content of chloroplast suspension was also estimated by dissolving 0.02 ml of chloroplast suspension in 2 ml of acetone (80%) and measuring absorbance at 652 nm. The rate of photosynthetic electron transport was expressed as micromoles of DCIP reduced per milligram chlorophyll per hour.

3.3.2.3. Ethylene release rate (ERR)

Young panicles of sterile and fertile TGMS lines were covered with 200 gauge thick polythene covers which were fitted with septum which was used to draw the

ethylene sample from the covers. Samples (2 ml) were drawn after 24 hours from the plastic covers and injected to Gas chromatograph (GC) and compared with standard sample for retention time, height of the peak, and area covered by the peak. The ethylene concentration was computed using the formula below as adopted by Sadasivam and Manickam (1996).

$$\text{Standard amount of ethylene (E) in } \mu \text{ mol} = \frac{0.0446 \times Z \mu \text{l}}{\text{Peak height in mm} \times \text{attenuation}}$$

Amount of ethylene evolved = E x Peak height of the sample in mm x attenuation

3.3.2.4. Nutrient analysis

3.3.2.4.1. Calcium content

The calcium content of leaves and young panicles was analysed by versanate titration method proposed by Piper (1956) and expressed in $\mu\text{g g}^{-1}$ on dry weight basis.

3.3.2.4.2. Copper content

The dried samples of leaves and young panicles were used for determination of copper content. It was estimated by Atomic absorption spectrophotometer method using triple acid extract (Lindsay and Norvell, 1978). The content of copper expressed in $\mu\text{g g}^{-1}$ of sample on dry weight basis.

3.3.2.4.3. Boron content

The content of boron in leaves and young panicle was estimated by the method proposed by Banuleos *et al.* (1992) using azomethine-H, and expressed in $\mu\text{g g}^{-1}$ of sample on dry weight basis.

3.3.3. Biochemical parameters

3.3.3.1. Protein content

The soluble protein content of the leaves was determined by measuring the colour developed by the reduction of Folin-Ciocalteu reagent by the amino acids like tyrosin and tryptophan of protein, following the method of Lowry *et al.* (1951) and expressed in mg g^{-1} FW.

3.3.3.2. Carbohydrate fractions

- i. Total carbohydrate was estimated using anthrone reagent as per the procedure of Hedge and Hofreiter (1962) after hydrolyzing the leaf samples with HCl and expressed in mg g^{-1} FW.
- ii. Starch content was estimated by extracting samples in perchloric acid 52 per cent (Hedge and Hofreiter, 1962) after removing sugars by repeated washing with methanol (80%) and the content was expressed in mg g^{-1} FW.

3.3.3.3. Total free amino acid content

Total free amino acid content was estimated from the ethanol extract by reaction with ninhydrin as per the method of Misra *et al.* (1975) and expressed in mg g^{-1} FW.

3.3.3.4. Total phenol content

Total phenol was estimated from the ethanol extract by reaction with Folin-Ciocalteu reagent as per the method of Malik and Singh (1980) and expressed in mg g^{-1} FW.

3.3.3.5. Proline content

Proline was estimated by selective extraction with 3 per cent aqueous sulphosalicylic acid after removing the interfering proteins. The chromophore developed,

while reacting with acid ninhydrin was estimated spectrophotometrically adopting the procedure of Bates *et al.* (1973) and expressed in mg g^{-1} FW.

3.3.3.6. Ascorbic acid content

Ascorbic acid content was determined by volumetric method as proposed by Sadasivam and Balasubramanian (1987).

One gram of the leaf was homogenized with 4 per cent oxalic acid and centrifuged at 5000 rpm for 10 min. The supernatant was made upto 25 ml. To 5 ml of the aliquot, added 10 ml of 4 per cent oxalic acid and titrated against 2, 6-dichlorophenol indophenol dye. 5 ml of working standard containing $100 \mu\text{g ml}^{-1}$ of ascorbic acid was also titrated for reference value for calculation. The amount of ascorbic acid was expressed as $\mu\text{g g}^{-1}$ FW.

3.3.3.7. Nucleic acids content (DNA and RNA)

The DNA and RNA contents were determined by the method proposed by Ashwell (1957).

3.3.3.8. IAA oxidase activity

IAA oxidase activity was estimated colorimetrically by adopting the method of Parthasarathy *et al.* (1970).

3.3.3.9. Nitrate reductase activity

Nitrate reductase activity was determined as per the method of Nicholas *et al.* (1976) and activity was expressed in μ moles of NO_2 produced $\text{h}^{-1} \text{g}^{-1}$ FW.

3.3.3.10. Ascorbic acid oxidase (AAO) activity

The AAO enzyme activity was determined by the method of Oberbacher and Vines (1963).

One gram of leaf sample was extracted with 0.1 M phosphate buffer (pH 6.5). A known volume of extract was added to the experimental cuvette containing 3 ml of substrate solution with ascorbic acid. The change in absorbance at 265 nm for every 30 seconds interval was measured for 5 min. From the linear phase of the reaction, the enzyme activity was calculated and expressed as enzyme units g^{-1} FW. All the operations were carried out at 0-5°C.

3.3.3.11. Malate dehydrogenase (MDH) activity (Sadasivam and Manickam, 1996)

Enzyme extract

The tissue was thoroughly macerated with acid washed sand in a pre-chilled pestle and mortar in grinding medium (1 ml/ 1 g tissue) containing 50 mM Tris-HCl (pH 8.0), 50 mM MgCl_2 , 5 mM 2-mercapto ethanol and 1 mM EDTA. The homogenate has passed through 4 layers of cheese cloth. The filtrate was centrifuged at 3000 g for 20 min at 5°C. The supernatant was used as enzyme source.

Materials

Oxaloacetic acid (5 μ mole : 66mg/50 ml distilled water).

Magnesium chloride (10 μ moles : 203.5 mg/50 ml distilled water)

Tris-HCl Buffer (0.1 M) pH 7.8.

NADH (0.4 μ mol : 5.32 mg/10 ml).

Procedure

All the reagents were pipetted as shown below.

S.No.	Reagents	Test (ml)	Blank (ml)
1.	Oxaloacetic acid	0.5	0.5
2.	MgCl ₂	0.5	0.5
3.	Tris-HCl (pH 7.8)	1.3	1.8
4.	Enzyme extract	0.2	0.2
5.	NADH	0.5	-

The spectrophotometer was set to zero absorbance at 340 nm without adding NADH in the test against blank in the reference cuvette. NADH was added quickly as possible into test, mixed well and recorded the initial absorbance. The absorbance was recorded every 30 seconds for at least 3 min.

The enzyme activity was calculated as follows with decrease in absorbance for one min

μ moles NADH oxidised per min per 0.2 ml enzyme extract

$$= \text{Absorbance decrease min}^{-1} \times 0.1613 \times 3 \text{ (volume of the reaction mixture in ml)}$$

3.3.3.12. Glutamate dehydrogenase (GDH) activity (Sadasivam and Manickam, 1996)**Enzyme extract**

One gram of the leaf tissue was extracted with 5 ml of 100 mM phosphate buffer pH 7.5 containing 1 mM disodium EDTA, 1 mM Dithioerythritol and 1 per cent polyvinyl pyrrolidone (PVP) and centrifuged at 10,000 g for 30 min at 4°C. The supernatant was used for enzyme assay.

Materials

Potassium phosphate buffer (pH 7.0)

2-oxoglutarate (0.1M : 14.6 g l⁻¹ distilled water).

NH₄Cl (1.0 M : 53.3 g l⁻¹ distilled water)

NADH (10 mg ml⁻¹)

The assay of NADH dependent GDH was proceeded as per the composition of assay given below.

S.No.	Reagents	NADH assay (ml)
1.	Potassium phosphate buffer pH 7.0	1
2.	2-oxoglutarate	0.3
3.	NH ₄ Cl	0.5
4.	NADH	0.12
5.	Enzyme extract	0.2
6.	Water	8

0.3 ml of water was added in the blank instead of 2-oxoglutarate. The reaction mixture was incubated at 37°C for 15-30 min. The change in absorbance was recorded at 340 nm. The amount of NADH oxidised was calculated from the molar extinction coefficient. Activities are expressed as nano mole NADH oxidised per min per ml enzyme source.

3.3.3.13. Acid phosphatase (AP) activity (Sadasivam and Manickam, 1996)

Enzyme extract

One gram of fresh tissue was homogenised in 10 ml of ice-cold 50 mM citrate buffer (pH 5.3) in a pre chilled pestle and mortar and filtered through four layers of cheese cloth. The filtrate was centrifuged at 10,000 g for 10 min. The supernatant was used as enzyme source.

Materials**Sodium hydroxide (0.085 N)**

0.85 g NaOH was dissolved in 250 ml water.

Substrate solution

EDTA 1.49 g, 0.84 g citric acid and 0.03 g p-nitrophenyl phosphate were dissolved in 100 ml water and pH was adjusted to 3.3.

Standard

69.75 mg p-nitrophenol was weighed and dissolved in 5 ml of distilled water (100 ml).

Procedure

Substrate solution (3 ml) was incubated at 37°C for 5 min. 0.5 ml enzyme extract and mixed was added thoroughly. From that 0.5 ml was removed immediately and mixed it with 9.5 ml of sodium hydroxide 0.085 N. This corresponds to zero time assay (blank). The remaining solution (substrate and enzyme) was incubated for 15 min at 37°C. 0.5 ml sample was drawn and mixed it with 9.5 ml sodium hydroxide solution. The absorbance of blank was measured and incubated the tubes at 465 nm. 0.2 to 1.0 ml (4 to 20 mM) of the standard was taken and diluted to 10.0 ml with NaOH solution. The colour intensity was read and the standard curve was drawn. Specific activity was expressed as m moles p-nitrophenol released per min per mg protein.

3.3.3.14. Catalase activity

Catalase activity was determined following the method of Luck (1974). One gram of the sample was extracted in 0.067 M phosphate buffer (pH 7.0). A known volume of the extract was added to the experimental cuvette containing 3 ml H₂O₂ - PO₄ buffer. The time taken for per cent change in absorbance (Δt), at 240 nm was recorded for

calculating the enzyme activity and expressed as enzyme units $\text{g}^{-1} \text{min}^{-1}$ tissue. All the operations were carried out at $0-5^{\circ}\text{C}$.

3.3.3.15. Peroxidase activity

Peroxidase activity was determined by adopting the method of Malik and Singh (1980).

One gram of leaf was extracted in 0.1 M phosphate buffer (pH 7.0). A known volume of the extract was added to an experimental cuvette containing 3 ml phosphate buffer and 0.05 ml guaiacol reagent and then 0.03 ml of H_2O_2 solution was added rapidly and the increase in absorbance at 436 nm was recorded. This Δt in minutes was used to calculate the enzyme activity. The enzyme activity was expressed as enzyme units per litre. All the operations were carried out at $0-5^{\circ}\text{C}$.

3.3.3.16. Superoxide dismutase (SOD) activity

SOD activity was determined by using nitroblue tetrazolium (NBT) salt as described by Beau Champ and Fridovich (1971) and expressed as enzyme units mg^{-1} protein min^{-1} . The reagents used in the enzyme assay are,

Reagents

Stock	Working solution	Total volume
1. Riboflavin 200 μM (100x) 3.8 mg/50 ml	2 μM	30 μl
2. Buffer 250 mM (5x) KH_2PO_4 3.402 g/100 ml K_2HPO_4 4.354 g/100 ml	50 mM	600 μl

3. Methionine 130 mM (10 x) 0.9698 g/50 ml	13 mM	300 µl
4. EDTA 10 mM (100x) 0.3722 g/100 ml	0.1 mM	30 µl
5. NBT 7500 µM (100x) (4.08 mg/5ml)	75µM	30µl
6. Enzyme extract in Phosphate buffer (pH 7.8)	-	50 µl
7. Distilled water		
i. for sample	-	1.96 ml
ii. Blank (NBT-enzyme extract)	2.04 ml	-
iii. Reference blank (Enzyme)	-	2.01 ml

The absorbance was measured at 560 nm

Calculations

Absorbance of reference blank = x

Absorbance of sample = y

$$\frac{x-y}{x} \times 100 = Z\% \text{ inhibition}$$

$$50 \text{ per cent inhibition} = \frac{Z}{50} = A \text{ unit}$$

i.e. 50 µl enzyme extract yielded A unit.

1000 µl yields $A/50 \times 1000 = B$ unit

$$\frac{B}{\text{Protein value}} = \text{SOD assay value (Enzyme units mg}^{-1} \text{ protein ml g}^{-1})$$

3.3.4. Molecular studies

3.3.4.1. Electrophoresis (SDS-PAGE)

SDS-PAGE analysis was done to identify the qualitative and quantitative differences of proteins under sterile and fertile condition as per the method of Laemmli (1970) with slight modifications as per the need given by Sadasivam and Manickam (1996).

A known quantity of leaf sample was homogenized in phosphate buffer (pH 7.0) in a pre chilled pestle and mortar and the homogenate was centrifuged at 10,000 rpm for 10 min and the supernatant was used for analysis.

Materials

Stock acrylamide solution

Acrylamide	30 g
Bis acrylamide	0.8 g
Water to	100 ml

Separating gel buffer (pH 8.8)

1.875 m Tris-HCl	22.7
Water	100 ml

Stacking gel buffer (pH 6.8)

0.6 M Tris-HCl	7.26 g
Water to	100 ml

Polymerising agents

APS	0.5g/20 ml, freshly prepared
TEMED	fresh from refrigerator

Electrode buffer (pH 8.2-8.4)

0.05 M Tris	12 g
0.192 M glycine	28.8 g
0.1% SDS	2 g
Water to	2 L

Sample loading buffer (5 X conc.)

Tris HCl buffer (pH 6.8)	5 ml
SDS	0.5 g
Sucrose	5 g
Mercaptoethanol	0.25 ml
Bromophenol blue (0.5% W/V)	1 ml
Water to	10 ml

SDS 10% solution (Stored at room temperature)

Protein stain solution

Coomassie brilliant blue R250	0.1 g
Methanol	40 ml
Acetic acid	10 ml
Water	50 ml

Destainer

as above without the dye

Procedure

Thoroughly cleaned glass plates and spacers were assembled properly and were clamped in an upright position on a gel casting unit with 2 per cent agar on bottom to seal the chamber leak proof between glass plates. 10-15 per cent gels were tried to get the better resolution.

Separating gel	15% gel	10% gel
Stock acrylamide	20 ml	13.3 ml
Tris-HCl (pH 8.8)	8 ml	8 ml
Water	11.4 ml	18.1 ml

Degassed for 3-5 min and added

APS (5%)	0.2 ml	0.2 ml
10% SDS	0.4 ml	0.4 ml
TEMED	20 μ l	20 μ l

The gel solution was poured in the chamber between glass plates carefully leaving 4cm from the top and a layer of distilled water was added on top of the gel. Allowed to polymerise for 30-60 min.

Stacking gel	4%
Stock acrylamide	1.35 ml
Tris-HCl (pH 6.8)	1 ml
Water	7.5 ml
APS (5%)	50 μ l
SDS (10%)	0.1 ml
TEMED	10 μ l

After removing water from the gel, stacking gel (4%) was added, comb was placed and kept for polymerizing (30-60 min).

After polymerization, the comb was taken out and the gel plate was removed from the casting unit to the electrophoresis apparatus. Concentration of the protein sample were adjusted to 50-200 μ g in a volume of 25-50 μ l, by mixing with sample loading buffer, boiled for 2-3 minutes for ensuring complete interaction between proteins and SDS. The sample was cooled and carefully injected to each well with a microsyringe. Then the apparatus was filled with electrode buffer in both tanks, cathode was connected at the top and anode at the bottom and turned the DC power pack to a constant current of 15 mA initially till the dye front crossed the stacking gel. Then the current was increased to 30 mA until the bromophenol blue reaches bottom of the gel (about 3 h). The gel was removed from the plates and immersed in staining solution overnight. After proper staining the gel was transferred to destainer with gentle shaking and changing the destainer frequently till appropriate visibility of the bands on gel. The gel was photographed.

3.3.4.2. Isozyme analysis

500 mg of leaf sample was homogenized in 1 ml phosphate buffer (pH 7.0) in a pre chilled pestle and mortar. The homogenate was centrifuged in a refrigerated centrifuge at 18,000 rpm for 15 min and the supernatant was used for analysis. Electrophoretic separation of isozyme was done with 7 per cent polyacrylamide gel without using SDS. Casting of the gel was done as described earlier, without using SDS anywhere. Loading of the samples was done in fridge and chilled electrode buffer without SDS was used. The power supply was set to a constant 15 mA current and the running of the gel was done inside refrigerator.

Staining procedure

3.3.4.2.1. Catalase (CAT) (Sadasivam and Manickam, 1996)

Staining solution

Solution A	:	Sodium thiosulphate (60 mM)- 30 ml
		H ₂ O ₂ 3% - 70 ml
Solution B	:	Potassium iodide (50 mM) - 100 ml
	:	Glacial acetic acid - 0.5 ml

Procedure

The gel was treated with solution A until bubbles appeared on the gel. Solution B was added after discarding solution A. Chromophore developed as orange / reddish orange bands.

3.3.4.2.2. Peroxidase (POX) (Sadasivam and Manickam, 1996)

Staining solution

Solution A

Bonzidine	- 100 mg
Acetic acid	- 0.5 ml
Acetone	- 0.5 ml
Ammonium chloride	- 6% w/v

Solution B

H₂O₂ - 30% v/v

Solution C

Glacial acetic acid - 7% v/v

Procedure

The gel was incubated in the dark in solution A. After 30 minutes solution B was added drop by drop till blue coloured bands appeared. The gel was then fixed with solution C and photographed.

3.3.4.2.3. Superoxide dismutase (SOD)

Beau Champ and Fridovich (1971)

Staining solution

250 mM potassium phosphate buffer (pH 7.8)	20 ml
10 mM EDTA	1 ml
TEMED	200 µl
Riboflavin	150 mg
NBT	15 mg
Water to	100 ml

The gel was incubated in dark for 30 minutes immersed in the staining solution. Then rinsed with water and exposed to bright sun light, till white bands appear on a bluish black background.

3.3.4.2.4. Esterase (EST) (Sadasivam and Manickam, 1996)**Staining solution**

100 mM naphthyl acetate	-	1 ml
Fast blue RR salt	-	0.1 g
20 mM phosphate buffer (pH 8.0)	-	to 100 ml

Stop solution		
Methanol	-	10 ml
Acetic acid	-	10 ml
Ethanol	-	5 ml
Distilled water	-	50 ml

Immediately after electrophoresis at 4°C the gel was incubated in staining solution for about 45 min at room temperature in dark. After appearance of enzyme bands, the reaction was stopped by adding the stop solution. The zones, where the enzymes are located in the gel were visualized by appearance of brown bands, and the gel was photographed.

3.3.4.2.5. Polyphenol oxidase (PPO) (Sadasivam and Manickam, 1996)

Procedure

Incubate the gel for 30 minutes in 0.1% -p-phenylene diamine in 0.1 M potassium phosphate buffer (pH 7.) followed by 10 mM catachol in the same buffer.

Nomenclature for isozyme

Vanden Berg and Wissman (1982) described the norms for the nomenclature of isozymes. The same norm was followed in this study. The acronyms for catalase, peroxidase, esterase superoxide dismutase and polyphenol oxidase were CAT, POX, EST, SOD and PPO respectively. The isozymes were given number corresponding to their relative mobility (Rm) related to the dye front, preceded by the standard acronym for the enzyme.

3.3.5. Morphological and anatomical studies

3.3.5.1. Microscope study of anther and pollen morphology

The anthers from fully developed spikelets before opening of the spikelets were collected and observed visually for fertility/sterility. Fertile anthers were yellow and

plumpy while the sterile ones looked white, shrivelled, irregular and kidney shaped. The anthers were crushed and pollen grains were collected in a clean glass slide. It was stained with iodine-potassium iodide (IKI) (Nelson, 1968) and observed under microscope. The pollen grains that were stained fully were considered as fertile while unstained shrivelled and empty pollen grains were considered as sterile.

3.3.5.2. Anther anatomy study through microtome sectioning

Paraffin sectioning of the anthers was done as described by Johanson (1940). For this purpose, anthers were collected before dehiscence from the TGMS lines both from normal condition and from those grown in the phytotron.

i) Fixation and washing

The anthers were fixed in formalin (40) per cent formaldehyde): Acetic acid: Alcohol (1:1:18) solution for 24 h in 4°C. The anthers were then transferred to 50 per cent, 60 per cent and 70 per cent ethanol each at one hour interval.

ii) Dehydration

The anthers were gradually dehydrated in tertiary butyl alcohol (TAB) series as shown in the Table.

Alcohol (%)	TBA (ml)	Ethanol (95%)	Distilled water (ml)	Duration (h)
50	1.0	4.0	5.0	2-3
70	2.0	5.0	3.0	12
85	3.0	5.0	1.5	12
95	4.5	5.0	1.0	12
100	7.5	2.5	0.0	12

After dehydration, the anthers were given two changes with complete TBA each at six hours interval. During the final change, a pinch of eosine was added to stain the specimen superficially so as to locate them inside the wax at embedding stage.

iii) Infiltration

The specimens were transferred to TBA: liquid paraffin (1:1) for 2-3 hours to a beaker containing three-fourth of solid wax (melting point 56-58°C). An additional quantity of TBA: Liquid paraffin mixture was again added to the specimen and the beaker was kept at 60-65°C for 12 hours. Two changes were given with pure paraffin wax at an interval of 6 hours each.

iv) Embedding

The specimen was embedded in glycerine coated paper boat and the wax was rapidly cooled in ice water to get wax blocks which were stored at 4°C.

v) Microtoming

Sections of 8-10 micron thickness were cut and the ribbons were stretched out by placing them in warm water (40°C) and received on a microslide coated with Haupt's adhesive. The slide were flooded with 4 per cent formalin and kept in warm plate (40°C) to get maximum stretching of the ribbon. The excess fluid was drained and the slides were shade dried for 10-12 hours in a dust free chamber.

vi) Staining and mounting

The slides were then passed through the following series.

Xylene-I, Xylene-II, Xylene:ethanol (1:1)-30 minutes each.

Ethanol-100%, 95%, 70, 50%, 30%, 10%, distilled water- 10 minutes each.

Ferrous ammonium sulphate (4%), 1% Erlich's hematoxylin (ripened for 30 days).

Washed in running tap water for 10-5 minutes.

Ethanol 10%, 50%, 70%, 100%,-5 minutes each.

Xylene: ethanol (1:1), Xylene I, Xylene II-5 minutes each.

The slides were mounted in DPX mountant and left undisturbed for 24 hours for examination.

3.4. Experiment IV. Chemical manipulation of fertility and sterility

3.4.1. Induction of sterility

3.4.1.1. Screening of chemicals and plant growth regulators for the induction sterility

An experiment was conducted to study the effect of various plant growth regulators and chemicals in inducing sterility in the glass house of the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore during winter months. The three selected TGMS lines were used in this experiment. The chemicals and plant growth regulators were tried with different concentrations, sprayed at 3 stages of panicle development. This is done to screen the effective chemicals and their concentrations and also to fix the correct stage of treatment to induce 100 per cent pollen sterility. Pollen sterility before anthesis was estimated by IKI staining.

Experimental details

Entries	:	TGMS 6, TGMS 16, TGMS 29	
Treatment	:	8	
T ₁	-	Control	
T ₂	-	Salicylic acid	- 400 ppm
T ₃	-	Salicylic acid	- 600 ppm
T ₄	-	Salicylic acid	- 800 ppm
T ₅	-	Maleic hydrazide	- 0.05%
T ₆	-	Maleic hydrazide	- 0.1%
T ₇	-	Ethrel	- 200 ppm
T ₈	-	Ethrel	- 400 ppm

Replication	:	Four
Design	:	FCRD
Stages of spray	:	PI stage
		PMC stage (5 days after PI)
		5 Days after PMC stage

Combinations of stages for treatment

Panicle initiation (PI) alone
 PI + Pollen mother cell formation (PMC) stage
 PI + PMC + 5 Days after PMC
 PMC alone
 PMC + 5 DA PMC
 5 DA PMC
 PI + 5 DA PMC

Observations recorded

1. Spikelet sterility percentage

3.4.1.2. Induction of sterility by spraying salicylic acid

3.4.1.2.1. Pot culture experiment (Plate 2)

Experimental details

Entries	:	3 (TGMS 6, TGMS 16, TGMS 29)
Treatment	:	2
T ₁	:	Control
T ₂	:	Salicylic acid (SA) 800 ppm
Replication	:	14
Design	:	FCRD
Stage of treatment	:	Panicle initiation stage

Observations recorded

1. Per cent Pollen sterility
2. Per cent spikelet sterility
3. Per cent selfed seed setting
4. Assessing the female fertility

After assessing the pollen sterility in TGMS lines, they were crossed by dusting pollen grains collected from the line C-20R and the panicles were covered with white paper cover. After maturity the panicles were harvested and per cent crossed seed setting was worked out.

3.4.1.2.2. Field experiment**Experiment details**

Entries : 2 (TGMS 6 TGMS 29)
 Treatment : 2
 T₁ : Control
 T₂ : Salicylic acid (800 ppm) (PI stage)
 T₃ : Salicylic acid (800 ppm) (PI + 5 DA PMC)
 Replication : 15
 Design : RBD
 Stage of spray : Panicle initiation stage
 5 days after PMC

Observations recorded

1. Per cent pollen sterility
2. Per cent crossed seed set (Clipping method)

Pollen sterility before anthesis was estimated by I-KI staining. After the confirmation of 100 per cent sterility, the spikelets were opened by clipping method and

dusted with pollen grains collected from the pollinator (var ADT 45) and covered by white paper covers. After maturity, per cent crossed seed setting was worked out.

3. Per cent crossed seed set (Rope pulling method)

This method is commonly used in hybrid seed production in rice. Here the panicles of fertile lines were pulled by ropes to shed their pollen grains over the spikelets of male sterile TGMS lines. After maturity, per cent crossed seed setting was worked out.

3.4.1.3. Effect of SA spray on biochemical parameters

Leaf samples were collected from control and treated plants at PI stage, 15 days after PI and at flowering stage for biochemical analysis.

1. Soluble protein content
2. Carbohydrate fractions
3. DNA content
4. RNA content
5. Proline content
6. Total phenol content
7. IAA oxidase
8. Ascorbic acid oxidase (AAO)
9. Catalase (CAT)
10. Peroxidase (POX)
11. Superoxide dismutase (SOD)

The contents of Protein, carbohydrates, nucleic acids, proline, phenols and all the enzyme activities were estimated as per the procedures described in the experiment III.

3.4.2. Induction of fertility

This experiment was conducted in the summer season to study the effect of different growth regulators and hormones in increasing seed set of TGMS lines, in the

glass house of the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore.

Experimental details

Entries : TGMS 6, TGMS 16, TGMS 29

Treatments : Six

T₁ - Control

T₂ - Ascorbic acid (100 ppm)

T₃ - IAA (50 ppm)

T₄ - BR (1 ppm)

T₅ - NAA (50 ppm)

T₆ - GA (50 ppm)

Replication - 4

Design - FCRD

The chemicals were sprayed at panicle initiation stage and the fertility was estimated by counting seed set.

STATISTICAL ANALYSIS

The data were analysed statistically by completely randomized block design. In experiments III and IV the data on TGMS lines only were subjected to statistical analysis and were compared with mean values of variety ADT-36. The experiment III was analysed by factorial CRD with 3 factors. The experiment on chemical manipulation was also analysed as per design.

Results

CHAPTER IV

EXPERIMENTAL RESULTS

The present investigation was carried out in the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore to understand the critical sterility temperature and the critical stage of thermosensitivity of selected TGMS lines using phytotran. The physiological, biochemical and molecular changes during fertility/sterility transformation was also studied to understand the exact mechanism responsible for male sterility in TGMS lines and to reveal the possibility of manipulating sterility or fertility using chemicals and growth regulators. The effect of chemicals on biochemical aspects of TGMS lines was also studied during induction of male sterility to know the causes for male sterility. The morphological changes of anthers and pollens were studied under fertile and sterile conditions. The results of various experiments were statistically analysed and the data are presented in appropriate tables with suitable figures.

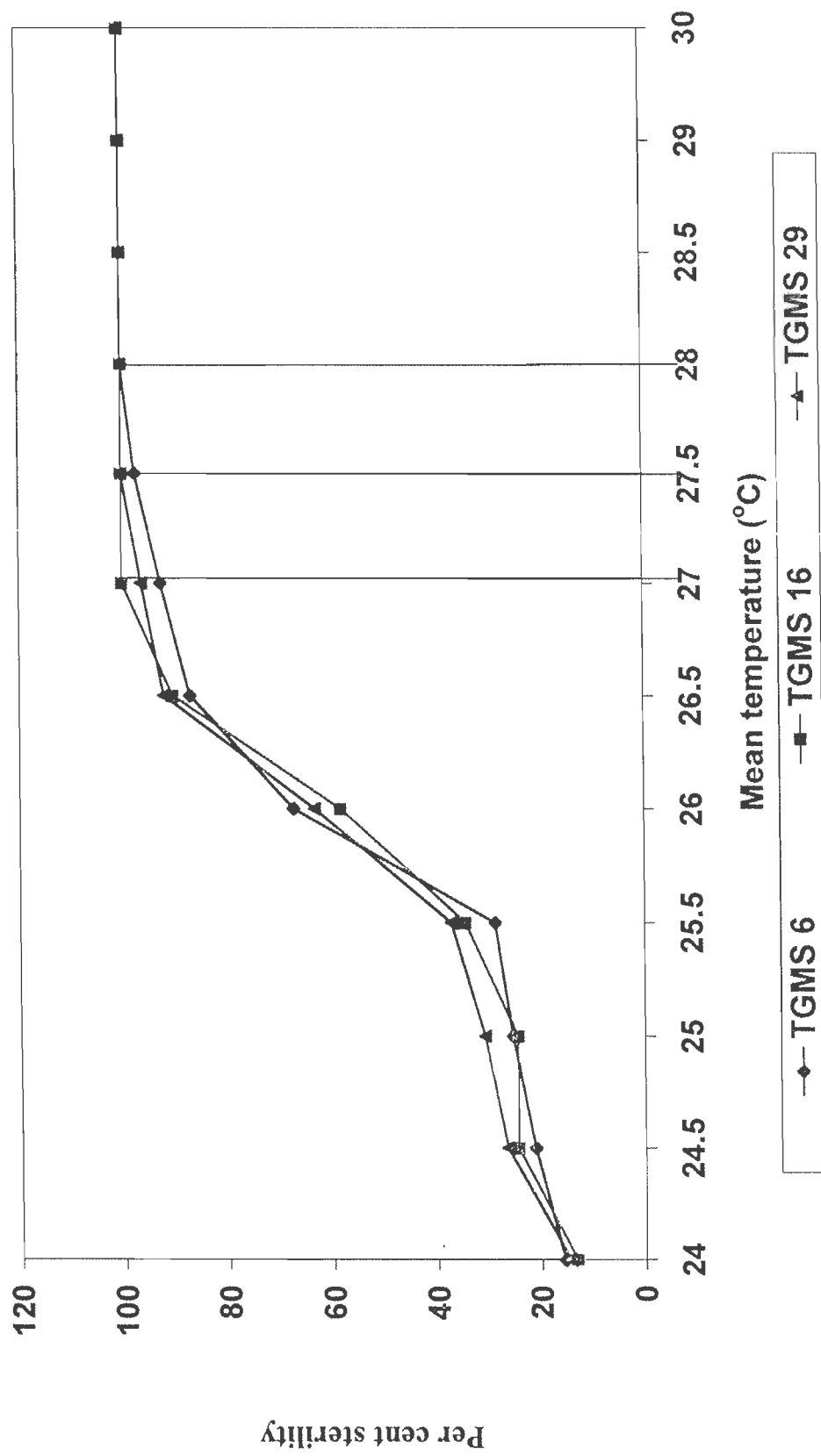
4.1. Critical sterility temperature (CST) of TGMS lines

An experiment was laid out to understand the critical sterility temperature of three TGMS lines by exposing them to different temperatures during panicle development stages. The per cent pollen sterility of TGMS 6, TGMS 16 and TGMS 29 at different daily mean temperatures is presented in Table 6. The per cent pollen sterility increased with increase in daily mean temperature and the genotypes behaved differently before they attained complete pollen sterility TGMS 6 attained 100 per cent sterility at 28.0°C (34/22°C), TGMS 16 at 27.0°C (33/21°C) and TGMS 29 at 27.5°C (34/21°C) (Fig.1). The anthers were pollen free above a daily mean temperature of 29°C. The results showed that there was varietal difference in the critical sterility temperature (CST) and that the CST of TGMS 6 was 28.0°C, TGMS 16 was 27.0°C and TGMS 29 was 27.5°C with 34.0, 33.0 and 34.0°C were the maximum temperatures respectively. All the TGMS lines

Table 6. Standardization of CFT and CST of TGMS lines

Temperature Max/ Min (⁰ C)	Mean Temperature(⁰ C)	Pollen sterility (%)			Mean
		TGMS 6	TGMS 16	TGMS 29	
36 / 24	30.0	100	100	100	100
35 / 23	29.0	100	100	100	100
34 / 23	28.5	100	100	100	100
34 / 22	28.0	100	100	100	100
34 / 21	27.5	97.3	100	100	99.1
33 / 21	27.0	92.6	100	96.3	96.3
32 / 21	26.5	87.0	90.3	92.0	89.7
32 / 20	26.0	67.3	58.3	63.3	62.9
31 / 20	25.5	28.6	34.3	37.0	33.3
30 / 20	25.0	25.3	24.3	30.6	26.7
29 / 20	24.5	21.0	24.3	26.3	23.8
28 / 20	24.0	15.3	13.0	14.3	14.2

Fig 1. Standardisation of CST for TGMS lines



showed their critical fertility point (CFT) at daily mean temperature below 24.0°C (28/20°C).

4.2. Critical stage of thermosenstivity

An experiment was conducted in mini phytotron with three TGMS lines to identify the critical stage of thermosenstivity. The TGMS lines were exposed to CST for different durations coinciding with various stages of panicle development to understand the critical stage of thermosenstivity for fertility alteration. All the TGMS lines were 100 per cent sterile when exposed from 0 to 30 days before heading (DBH), 5-25 DBH and 10-25 DBH. Exposing the TGMS lines to CST from 5-20 DBH and 10-20 DBH recorded 90 and 82 per cent sterility respectively whereas 10 days exposure in the later (20-30 DBH) or early stage (5-15 DBH) of panicle development did not increase the pollen sterility percentage of TGMS lines (Table 7). The treatment and the interaction effects were highly significant. The results indicated that the critical stage of thermosenstivity for fertility alteration in TGMS lines was 10 to 25 days before heading, as it was the minimum period of exposure to CST to get complete pollen sterility.

4.3. Physiological and Biochemical changes during fertility alteration

4.3.1. Physiological changes

4.3.1.1. Gas exchange measurements

Table 8 presents the changes in major gas exchange parameters *viz.*, photosynthetic rate (P_n) transpiration rate (E) and stomatal diffusive resistance (r_s) of TGMS lines under fertile and sterile conditions. Photosynthetic rate reduced upto 25 per cent in sterile condition compared to fertile condition (Fig.2). The highest percentage of reduction was observed in TGMS 29 (30.0%). There was no significant variation between lines, but the treatmental effect was highly significant. The per cent change in transpiration rate and stomatal resistance was 9.6 per cent and 12.3 respectively

Table 7. Pollen sterility (%) under different thermosensitive stages during pollen development

Treatment (Days before heading)	TGMS 6	TGMS 16	TGMS 29	Mean
0-30	100.00	100.00	100.00	100.00
5-25	100.00	100.00	100.00	100.00
5-20	90.73	90.16	92.20	91.01
5-15	25.46	27.0	31.93	28.13
10-20	78.70	83.93	84.46	82.36
10-25	100.00	100.00	100.00	100.00
20-30	25.30	32.16	36.20	31.22
Mean	71.61	74.47	76.61	
	T	L	L x T	
CD (P=0.05)	1.576	1.032	2.731	

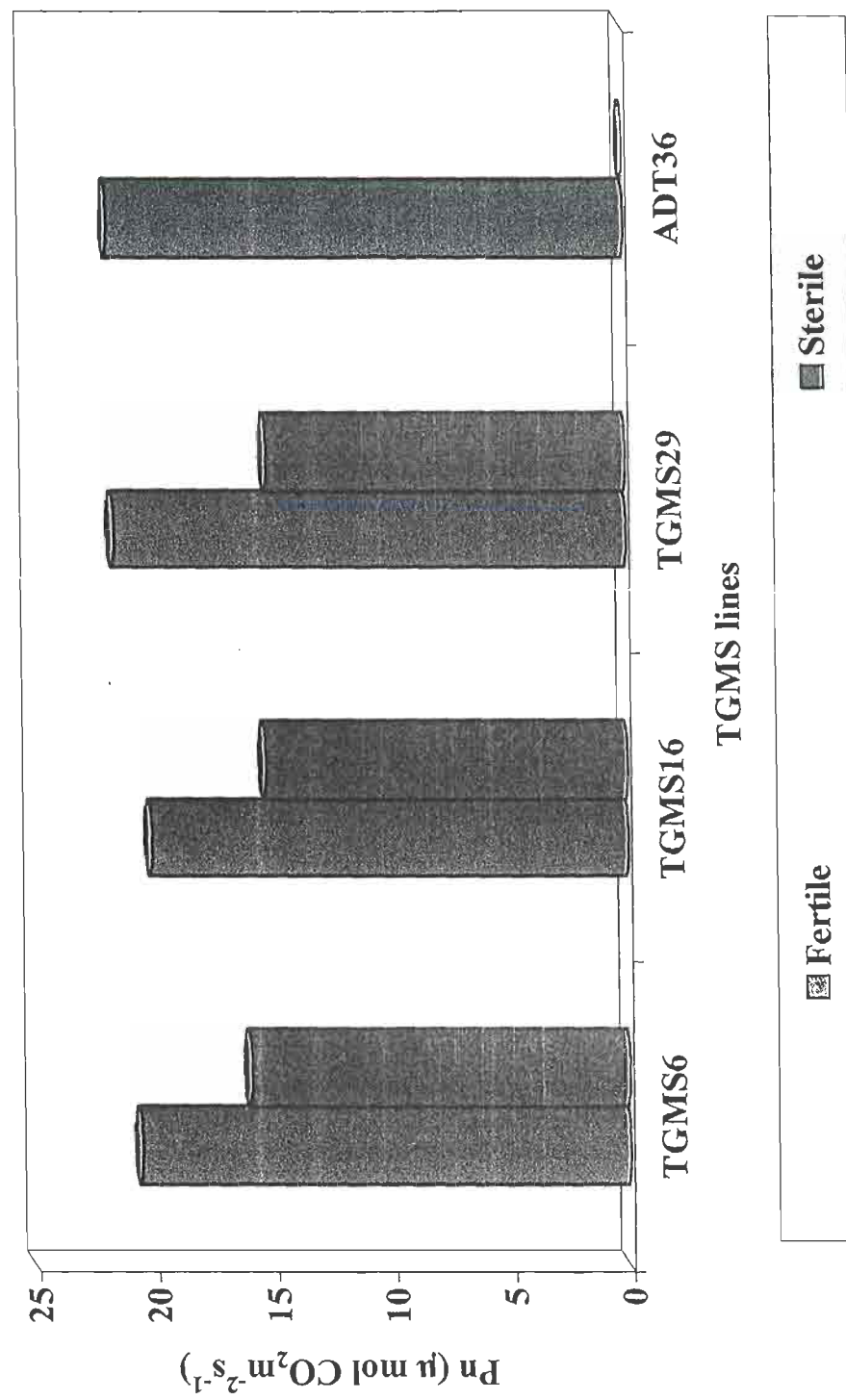
Table 8. Gas exchange measurements of TGMS lines under fertile and sterile conditions

Parameters	TGMS Lines	Fertile	Sterile	% Change
Photosynthetic rate (P _n) (μ mol CO ₂ m ⁻² s ⁻¹)	TGMS6	20.5	15.9	23.0
	TGMS16	20.0	15.2	24.0
	TGMS29	21.5	15.1	30.0
	ADT36	21.7	-	-
	Mean	20.6	15.4	25.6
Transpiration rate (E) (μ g H ₂ O m ⁻² s ⁻¹)	TGMS6	8.3	7.1	15.0
	TGMS16	7.1	6.8	5.0
	TGMS29	8.5	7.8	9.0
	ADT36	8.3	-	-
	Mean	7.9	7.2	9.6
Diffusive resistance (r _s) (cm s ⁻¹)	TGMS6	0.58	0.65	11.0
	TGMS16	0.63	0.70	10.0
	TGMS29	0.55	0.65	16.0
	ADT36	0.61	-	-
	Mean	0.58	0.66	12.3

CD (P=0.05)

	T	L	T x L
P _n	1.06	1.28	1.76
E	0.41	0.37	0.54
r _s	0.35	0.46	0.62

Fig.2 Photosynthetic rate of TGMS lines under fertile and sterile conditions



and was lesser when compared to the per cent change in P_n . The increase in r_s was associated with corresponding decrease in E . The results indicated that the P_n and E decreased but r_s increased under sterile condition in TGMS lines. The reduction in photosynthetic rate was not related to the increase in diffusive resistance, indicating a possible reduction in the mesophyll conductance due to change in energy transfer and enzyme activities.

4.3.1.2. Hill reaction

Table 9 exhibits the Hill reaction rate of TGMS lines under fertile and sterile conditions in comparison with a normal variety ADT 36. Hill reaction rate reduced significantly under sterile condition (24.0%) with only a marginal genotypic variation. The normal variety ADT 36 recorded the highest value for hill reaction rate ($53.47 \mu \text{ moles DCIP reduced mg}^{-1} \text{ chlorophyll h}^{-1}$) which was on par with TGMS lines under fertile condition (Fig. 3). The results indicated that the photochemical reaction was altered under sterile condition.

4.3.1.3. Ethylene release rate

The data on ethylene peak, area covered and ethylene concentration with the retention time of 51 seconds in young panicles of TGMS lines under fertile and sterile conditions are presented in Table 10.

The ethylene peak and area covered between the peak and attenuation stage showed a significant difference between fertile and sterile panicles. The highest peak and area recorded by sterile panicles of TGMS 29 (0.399 mV and $5.531 \text{ mV sec}^{-1}$) followed by TGMS 16 (0.378 mV and $5.479 \text{ mV sec}^{-1}$) under high temperature induced sterile condition. The fertile panicles recorded ethylene peak as 0.106 , 0.127 and 0.138 mV for TGMS 6, TGMS 16 and TGMS 29 respectively.

Table 9. Hill reaction rate of TGMS lines under fertile and sterile conditions

TGMS Lines	μ moles DCIP reduced mg^{-1} Chlorophyll hr^{-1}		
	Fertile	Sterile	% reduction
TGMS6	52.37	41.11	22.0
TGMS16	48.40	40.23	17.0
TGMS29	50.45	39.30	33.0
ADT36	53.47	-	-
Mean	50.40	40.21	24.0
	L	T	L xT
CD (P=0.05)	0.982	0.767	1.548

Fig.3 Hill reaction rate of TGMS lines under fertile and sterile conditions

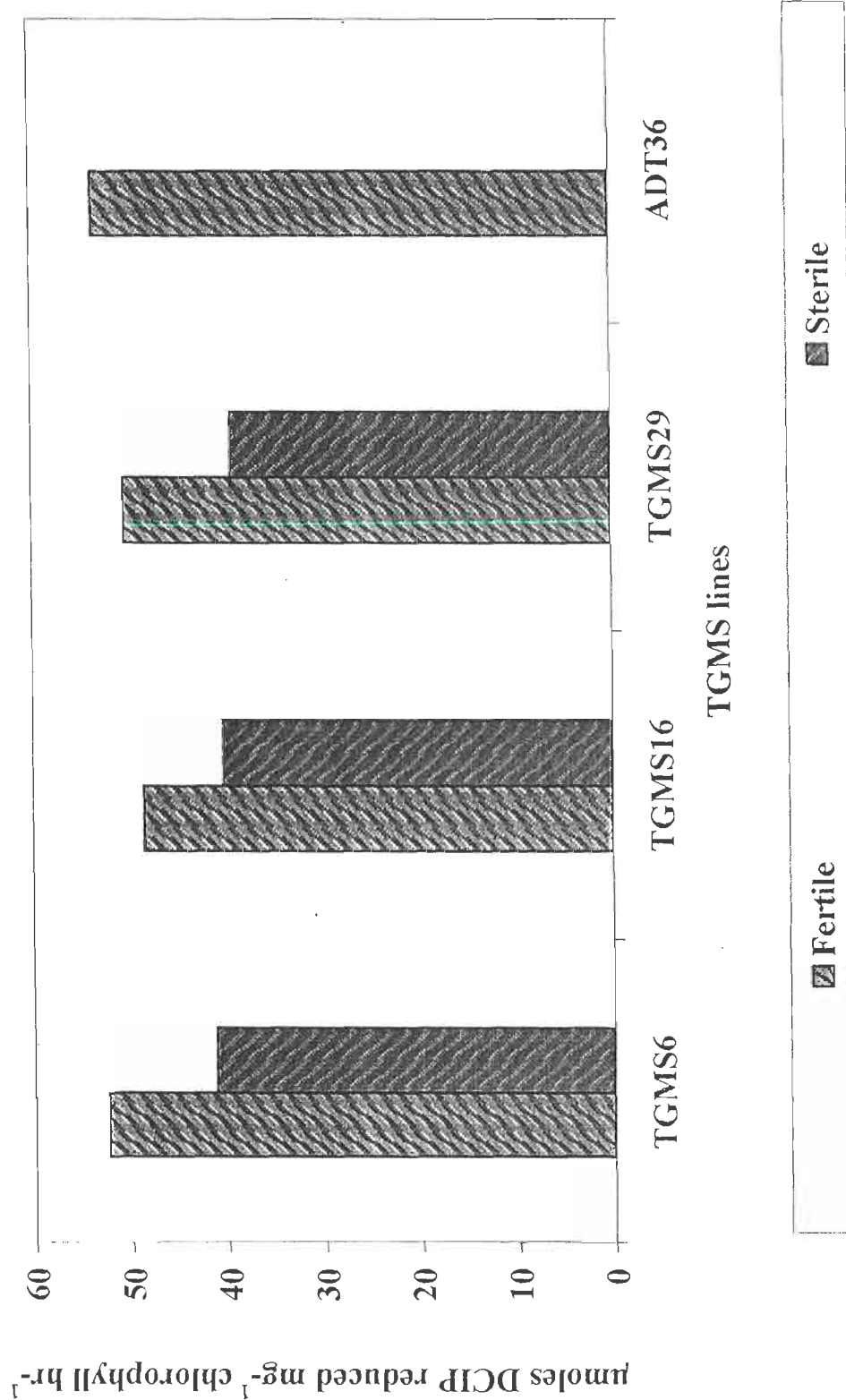


Table 10. Ethylene release rate (ERR) in panicles of TGMS lines under fertile and sterile condition ($\mu\text{mol min}^{-1}$)

TGMS lines	Height of peak (ml)		Area (ml s^{-1})		Ethylene concentration (μmol)	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
TGMS 6	0.106	0.263	1.203	2.690	2.047	3.787
TGMS 16	0.127	0.378	1.629	5.479	2.268	5.645
TGMS 29	0.138	0.399	1.990	5.531	2.403	5.784
ADT 36	0.081	-	1.012	-	1.723	-
Mean	0.123	0.346	1.607	4.566	2.239	5.072

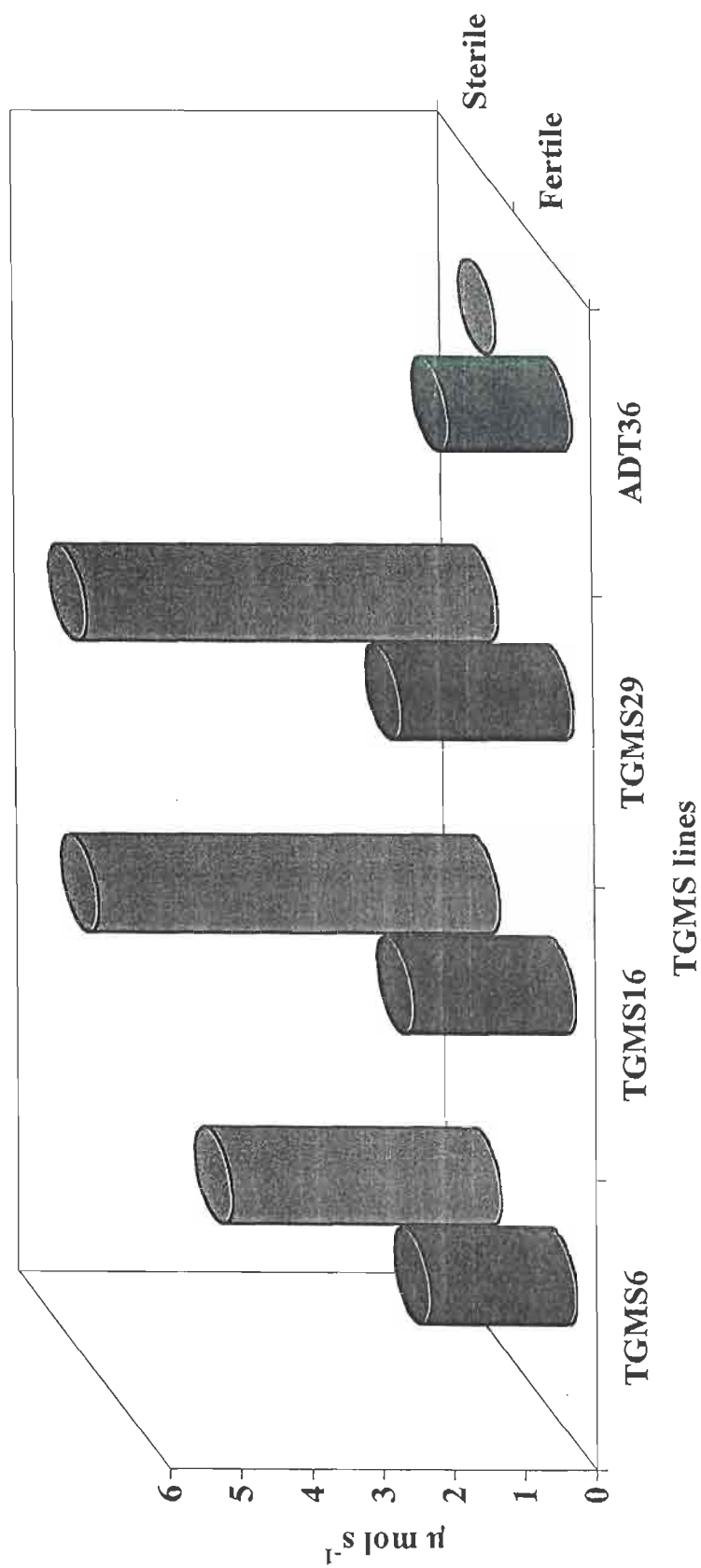
CD (P=0.05)

L 0.164
T 0.116
L x T 0.233

0.017
0.012
0.024

0.441
0.312
0.624

Fig.4 E thylene release rate of TGMS lines under fertile and sterile conditions



The concentration of ethylene released from young panicles also showed a significant difference between fertility and sterility. The fertile panicles of TGMS lines recorded ethylene concentration from 2.047 μ mol in TGMS 6 to 2.403 μ mol in TGMS 29 and in sterile panicles it was from 3.787 μ mol in TGMS 6 to 5.784 μ mol in TGMS 29 (Fig. 4). ADT 36 recorded the lowest values for ethylene peak (0.081 mV), area covered (1.012 mV sec⁻¹) and concentration (1.723 μ mol) under fertile condition. In this experiment, the treatment and the interaction effects were significant.

4.3.1.4. Nutrient content

The nutrients (Ca, Cu and B) status of leaves and panicles of TGMS lines in comparison with a normal variety ADT 36 is presented in Table 11. The secondary nutrient calcium and the micronutrients, copper and boron contents were reduced significantly in leaves (Fig. 5) and young panicles of sterile TGMS lines compared to fertile TGMS lines. There was no significant variation in Ca, Cu, and B contents between genotypes. The nutrient status of ADT 36 was on par with fertile TGMS lines. From this result, it is inferred that the status of calcium, copper and boron, which are playing major role in reproductive growth of plants, was changed in TGMS lines during fertility alteration.

4.3.2. Biochemical changes

4.3.2.1. Soluble protein content

Soluble protein content in leaves and young panicles of TGMS lines at different thermosensitive stages during fertility alternation is presented in Table 12 and 13. Soluble protein showed significant reduction under sterile condition compared to fertile TGMS lines. The per cent reduction increased from 10.66 in stage 2 to 39.33 in stage 5 in leaves (Fig. 6) and from 37.33 in 15 DBH to 40.0 in flowering stage in young panicles under sterile condition. The protein content of TGMS lines under fertile condition and in ADT 36 increased continuously over stages in both leaves and young panicles. However in

Table 11. Nutrient status of TGMS lines under fertile and sterile conditions

TGMS lines	Calcium ($\mu\text{g g}^{-1}$)				Copper ($\mu\text{g g}^{-1}$)				Boron ($\mu\text{g g}^{-1}$)			
	Leaves		Panicles		Leaves		Panicles		Leaves		Panicles	
	F	S	F	S	F	S	F	S	F	S	F	S
TGMS6	201	165	170	140	104	81	90	65	38	22	31	16
TGMS16	198	162	173	132	110	80	90	69	36	23	30	15
TGMS29	208	162	186	126	106	81	92	66	39	22	32	15
ADT36	203	-	180	-	119	-	93	-	38	-	31	-
Mean	202.3	163.0	176.3	132.6	106.6	80.6	90.6	66.6	37.6	22.3	31.0	15.3

CD (P=0.05) L N T L x N L x T T x N L x N x T

3.416 2.902 3.021 6.124 5.772 5.432 7.462

Fig.5 Nutrient status of TGMS lines under fertile and sterile conditions

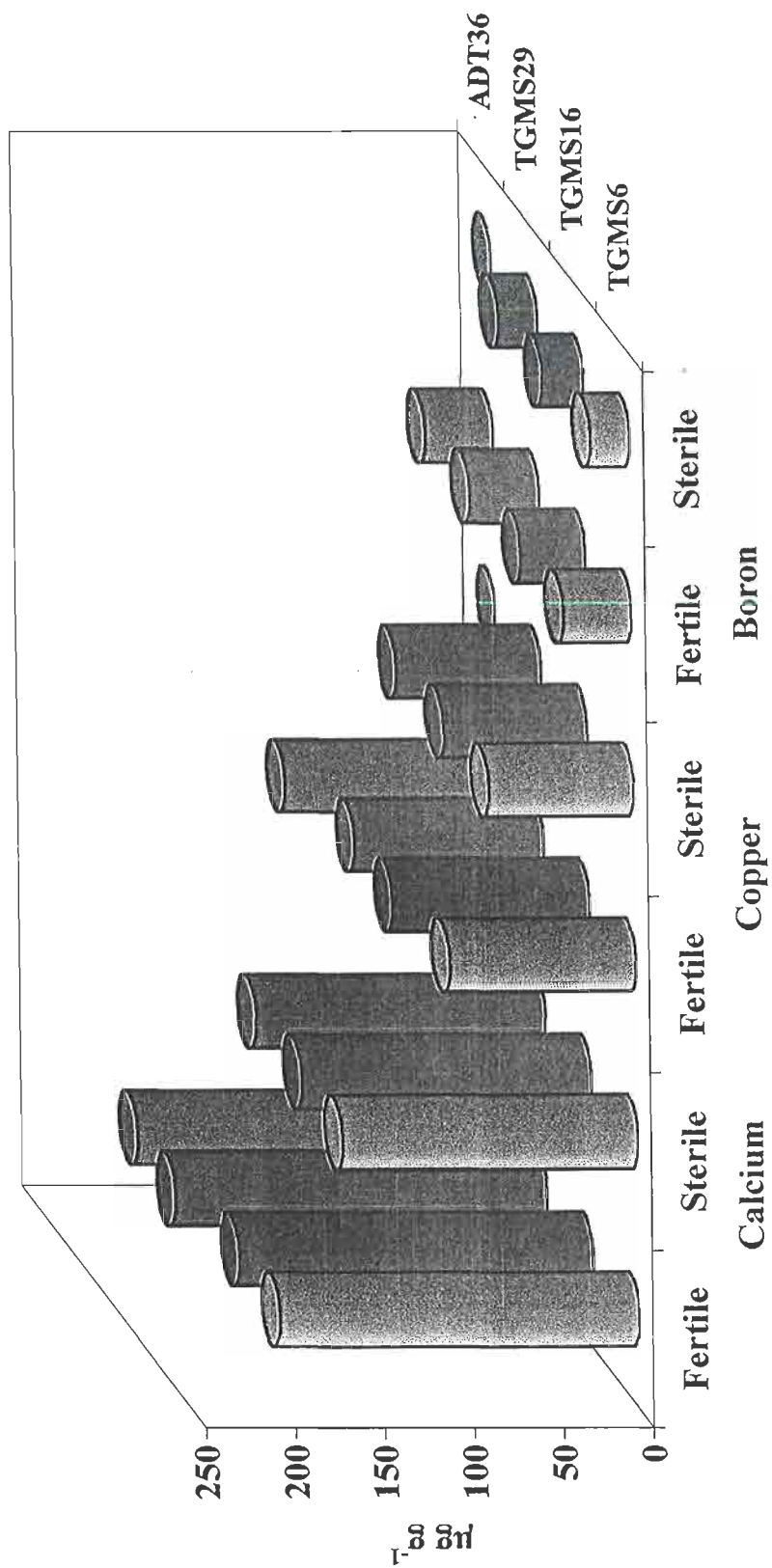


Table 12. Soluble protein content (mg g^{-1}) in leaves of TGMS lines at different thermosensitive stages during fertility alteration

TGMS lines	S1 (25 DBH)			S2 (20 DBH)			S3 (15DBH)			S4 (10 DBH)			S5 (5 DBH)		
	F	S	% change	F	S	% change	F	S	% change	F	S	% change	F	S	% change
TGMS 6	7.40	7.27	-	8.52	7.55	12	10.67	8.00	26	12.72	7.90	38	12.97	8.03	39
TGMS 16	7.00	6.97	-	8.45	7.92	7	10.80	8.42	23	11.95	7.80	35	12.82	8.20	37
TGMS 29	7.85	7.72	-	8.87	7.75	13	11.57	8.05	31	12.07	7.52	38	13.20	7.75	42
ADT 36	7.53	-	-	8.66	-	-	11.82	-	-	13.26	-	-	14.78	-	-
Mean	7.41	7.15	-	8.59	7.74	10.66	11.01	8.15	26.6	12.24	7.77	37.0	12.99	7.99	39.3

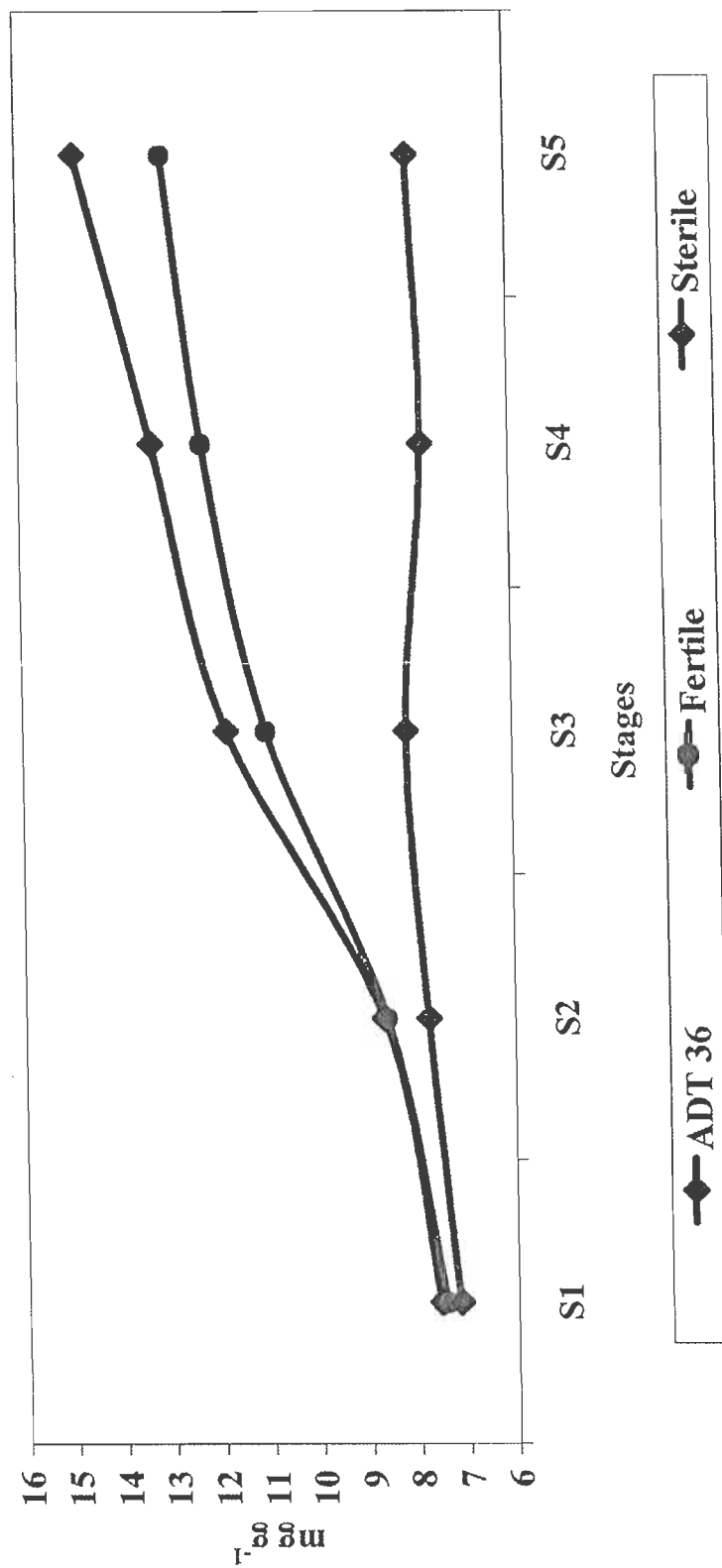
L S T L x S L x T S x T L x S x T
 CD ($P=0.05$) 0.302 0.390 0.247 0.676 0.428 0.552 0.957

Table 13. Soluble protein content (mg g^{-1}) in young panicles of TGMS lines under fertile and sterile conditions

TGMS lines	15 DBH				Flowering			
	Fertile	Sterile	% change		Fertile	Sterile	% change	
TGMS 6	2.77	1.75	37		4.31	2.82	35	
TGMS 16	2.82	1.79	37		4.82	3.08	37	
TGMS 29	2.98	1.87	38		4.88	2.56	48	
ADT 36	2.62	-	-		4.37	-	-	
Mean	2.85	1.80	37.3		4.67	2.82	40.0	

L S T L x S L x T S x T L x S x T
 CD ($P=0.05$) 0.028 0.023 0.021 0.042 0.033 0.040 0.057

Fig.6 Soluble protein content of TGMS lines under fertile and sterile conditions



sterile TGMS lines there was no significant increase in soluble protein content. Among the TGMS lines, TGMS 29 had higher protein content in all stages under fertile condition in both leaves and young panicles (7.85 mg g^{-1} to 13 mg g^{-1} and 2.98 mg g^{-1} to 4.88 mg g^{-1}) but ADT 36 recorded the highest soluble protein content in all the stages. Results indicated that there was a reduction of soluble protein content in leaves and young panicles of TGMS lines under sterile condition and the S, T, L x S, L x T and L x S x T interactions were significant from stage 2 onwards in leaves and 15 DBH and flowering stages in young panicles.

4.3.2.2. Total carbohydrate content

Total carbohydrate content in leaves and young panicles of TGMS lines under fertile and sterile conditions during different stages of panicle development is presented in Tables 14 and 15. The content reduced significantly under sterile condition and the percentage of difference between fertile and sterile increased from stage 2 (8.6%) to stage 5 (16.0%) in leaves (Fig. 7) and 23.6 per cent in 15DBH to 16.3 per cent in flowering stage in young panicles. In fertile lines, the carbohydrate content increased continuously similar to ADT 36, which recorded higher carbohydrate content compared to TGMS lines in all the stages of panicle development in leaves (76.52 mg g^{-1} to 101.22 mg g^{-1}) and in young panicles (43.73 mg g^{-1} and 71.30 mg g^{-1}) but under sterility, the content remained constant in all the stages. Among the TGMS lines, TGMS 16 recorded the highest carbohydrate content in all the stages under fertile and sterile conditions with values ranging from 73.87 mg g^{-1} to 95.87 mg g^{-1} from stage 1 to stage 5 respectively in leaves and 43.17 mg g^{-1} in 15 DBH and 69.12 mg g^{-1} in flowering stage in young panicles. Results suggested that there was significant difference in carbohydrate content between sterile and fertile TGMS lines with minimal genotypic variation. The treatment effect was seen from stage 2 indicating an impaired carbohydrate synthesis due to high temperature

Table 14. Total carbohydrate content (mg g^{-1}) in leaves of TGMS lines at different thermosensitive stages during fertility alteration

TGMS lines	S1			S2			S3			S4			S5		
	F	S	% change	F	S	% change	F	S	% change	F	S	% change	F	S	% change
TGMS 6	71.50	70.97	-	80.12	73.15	9	91.10	78.00	15	94.12	79.02	16	94.65	79.70	16
TGMS 16	73.87	72.75	-	82.87	75.22	10	92.85	79.37	15	95.25	79.30	17	95.87	80.10	16
TGMS 29	70.07	68.92	-	79.22	73.85	7	88.17	78.22	12	92.45	78.82	16	92.80	78.92	16
ADT 36	76.52	-	-	84.17	-	-	93.32	-	-	99.31	-	-	101.2	-	-
Mean	71.81	70.88	-	80.73	74.06	8.6	90.70	78.53	14.0	93.94	79.04	16.3	94.4	79.57	16.0

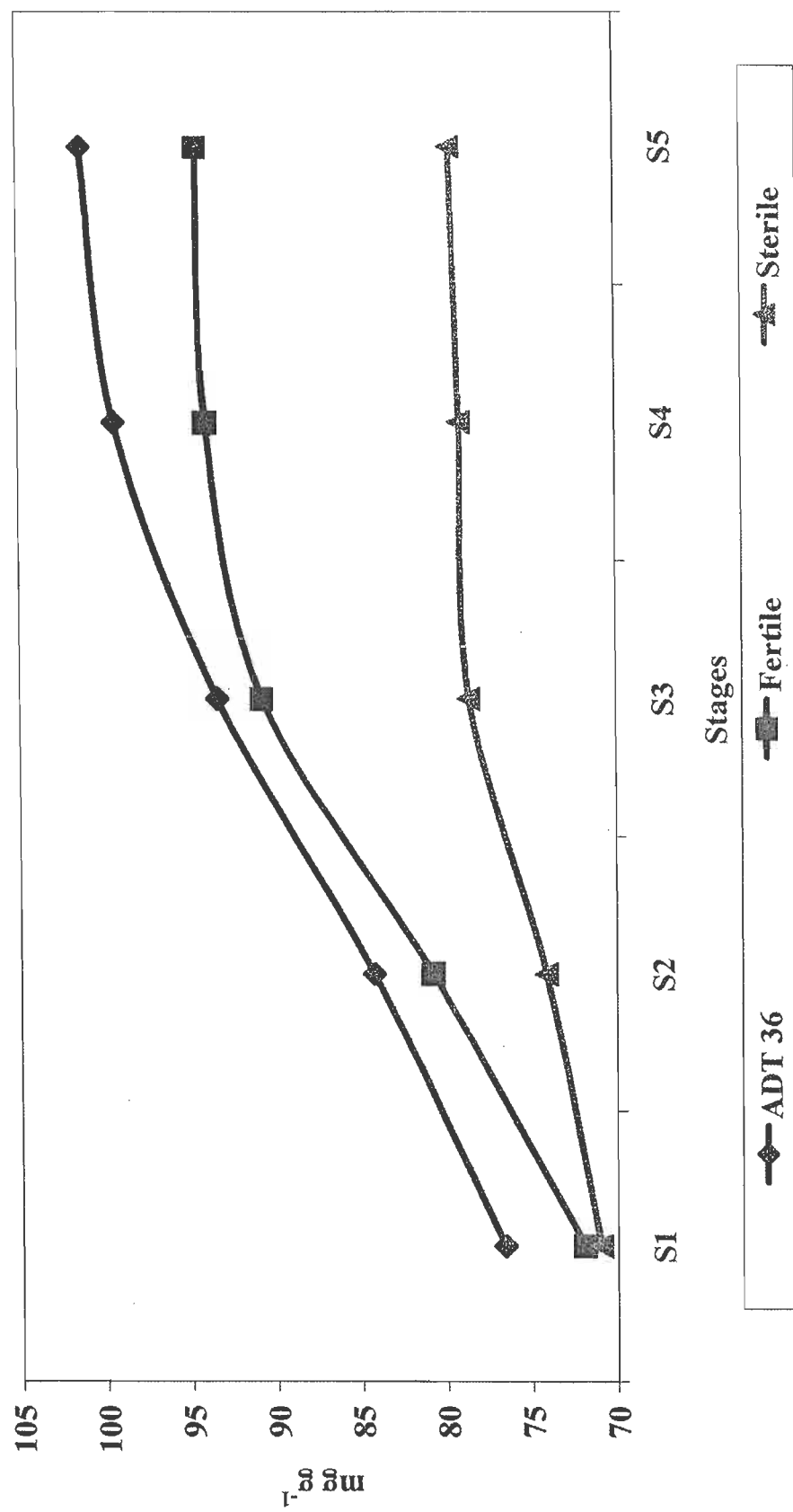
L S T L x S L x T S x T L x S x T
 CD ($P=0.05$) 0.166 0.214 0.135 0.372 0.235 0.303 0.526

Table 15. Total carbohydrate content (mg g^{-1}) in young panicles of TGMS lines under fertile and sterile condition

TGMS lines	15 DBH				Flowering			
	Fertile		Sterile		Fertile		Sterile	
TGMS 6	42.38		34.66	20	67.30		54.80	20
TGMS 16	43.17		30.62	31	69.12		58.41	16
TGMS 29	41.44		31.41	25	58.40		51.33	13
ADT 36	43.73		-	-	71.30		-	-
Mean	42.33		32.23	23.6	64.94		54.8	16.3

L S T L x S L x T S x T L x S x T
 CD ($P=0.05$) 0.210 0.172 0.162 0.297 0.243 0.294 0.421

Fig.7 Total carbohydrate content of TGMS lines under fertile and sterile conditions



induced sterility and the L x S, L x T, S x T and L x S x T interactions were significant in all the stages of panicle development.

4.3.2.3. Starch content

The data on starch content of the TGMS lines under fertile and sterile conditions at different stages in comparison with normal variety (ADT 36) are given in Tables 16 and 17. Under sterile condition, TGMS lines accumulated significantly higher starch compared to fertile condition in leaves. The mean values from stage 1 to stage 5 in leaves were from 30.19 mg g⁻¹ to 34.46 mg g⁻¹ and 32.49 mg g⁻¹ to 47.37 mg g⁻¹ under fertile and sterile conditions respectively (Fig. 8). But in young panicles the accumulation of starch was more under fertile condition (14.91 mg g⁻¹ to 24.65 mg g⁻¹) compared to sterile condition (12.34 mg g⁻¹ to 21.44 mg g⁻¹) in TGMS lines. The normal variety (ADT 36) behaved same as that of fertile TGMS lines in starch accumulation. The increase of starch in sterile lines from stage 1 to stage 5 was 7.0 per cent and 28.33 per cent respectively and it was 19.0 per cent in 15 DBH and 14.0 per cent in flowering stage in fertile panicles.

All the interactions were significantly different in all the stages except in first stage. The results suggested that starch accumulated in leaves of sterile TGMS lines and young panicles of fertile TGMS lines indicating poor translocation of starch under sterile condition compared to fertile condition. The varietal difference was significant, but it was marginal and the treatment effect was however, highly significant.

4.3.2.4. Total free amino acid content

Tables 18 and 19 show the total free amino acid content of TGMS lines under fertile and sterile conditions at different stages of panicle development in comparison with normal variety (ADT 36). There exhibited a significant reduction in amino acid accumulation under sterile condition in leaves from 22.0 per cent in stage 2 to

Table 16. Starch content (mg g^{-1}) in leaves of TGMS lines at different thermosensitive stages during fertility alteration

TGMS lines	S1			S2			S3			S4			S5		
	F	S	% change	F	S	% change	F	S	% change	F	S	% change	F	S	% change
TGMS 6	29.02	32.17	10	33.40	35.32	6	35.92	39.75	11	32.30	44.05	28	32.70	46.35	31
TGMS 16	31.60	33.50	7	35.55	38.60	8	38.20	40.85	5	33.95	45.08	27	35.25	47.75	26
TGMS 29	30.05	31.82	4	34.45	36.32	6	38.87	42.05	10	35.37	45.72	23	35.45	48.02	28
ADT 36	32.11	-	-	35.02	-	-	35.27	-	-	35.82	-	-	35.73	-	-
Mean	30.19	32.49	7.0	34.46	36.81	6.6	37.66	40.88	8.6	33.84	44.93	26.0	34.46	47.37	28.3

L S T L x S L x T S x T L x S x T
 CD (P=0.05) 0.246 0.317 0.201 0.348 0.449 0.778

Table 17. Starch content (mg g^{-1}) in young panicles of TGMS lines under fertile and sterile conditions

TGMS lines	15 DBH				Flowering			
	Fertile	Sterile	% change		Fertile	Sterile	% change	
TGMS 6	15.31	12.21	21		22.16	20.17	10	
TGMS 16	14.16	12.76	15		25.33	22.74	12	
TGMS 29	15.27	12.08	21		26.47	21.43	20	
ADT 36	15.56	-	-		26.73	-	-	
Mean	14.91	12.34	19.0		24.65	21.44	14.0	

L S T L x S L x T S x T L x S x T
 CD (P=0.05) 0.232 0.196 0.192 0.339 0.326 0.268 0.465

Fig.8 Starch content of TGMS lines under fertile and sterile conditions

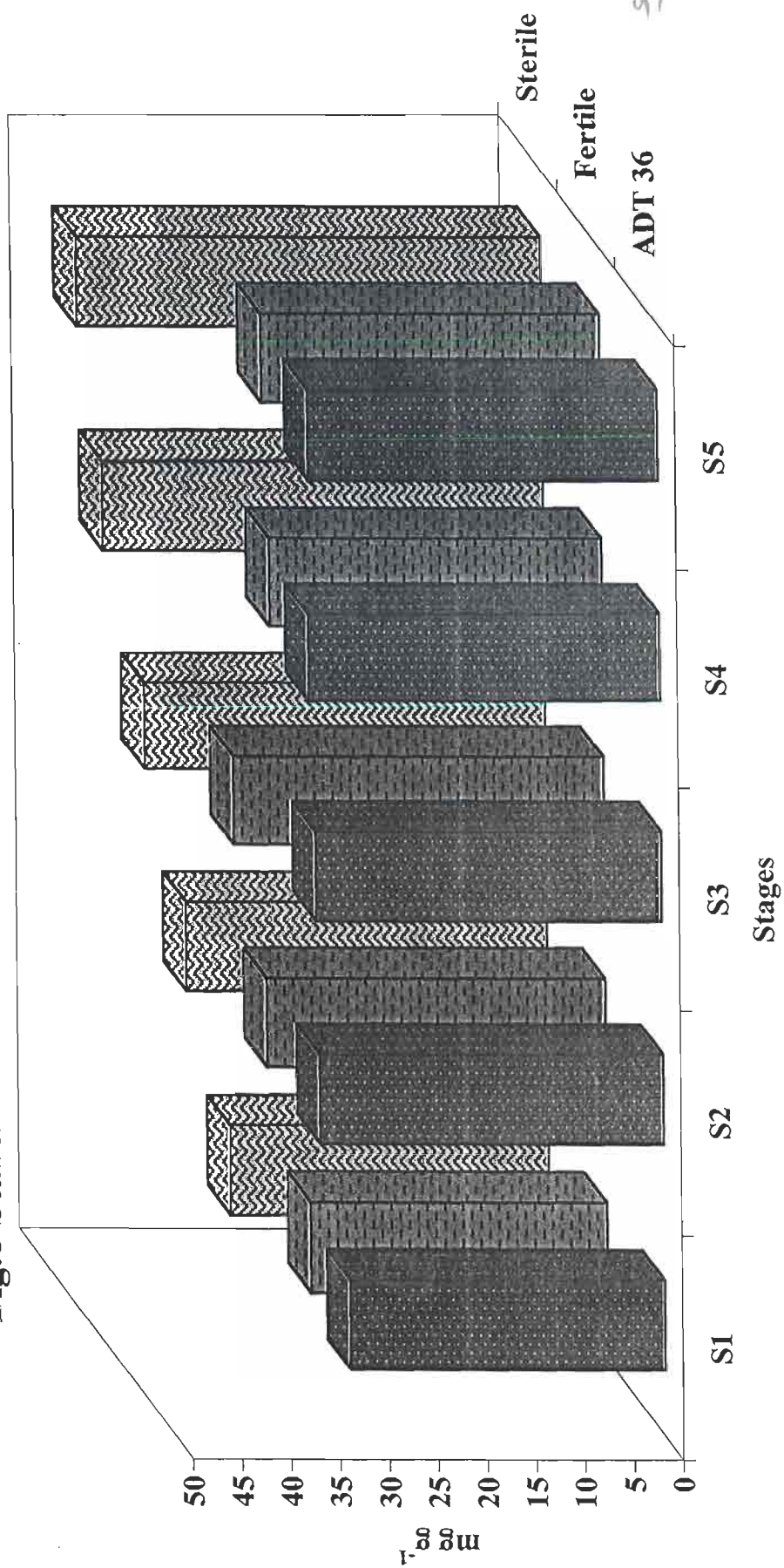


Table 18. Total free amino acid content (mg g^{-1}) in leaves of TGMS lines at different thermosensitive stages during fertility alteration

TGMS lines	S1			S2			S3			S4			S5		
	F	S	% change	F	S	% change	F	S	% change	F	S	% change	F	S	% change
TGMS 6	3.13	3.08	-	3.52	2.85	20	3.86	2.33	40	3.92	2.06	49	3.85	1.99	49
TGMS 16	2.52	2.29	-	2.97	2.20	25	3.72	2.11	44	3.76	2.20	41	3.86	1.92	51
TGMS 29	3.14	3.07	-	3.70	2.93	21	3.94	2.54	37	4.07	2.19	45	4.09	2.04	48
ADT 36	2.79	-	-	3.38	-	-	3.88	-	-	4.02	-	-	3.93	-	-
Mean	2.93	2.81	-	3.39	2.79	22.0	3.84	2.32	40.3	3.91	2.15	45.0	3.93	2.01	49.3

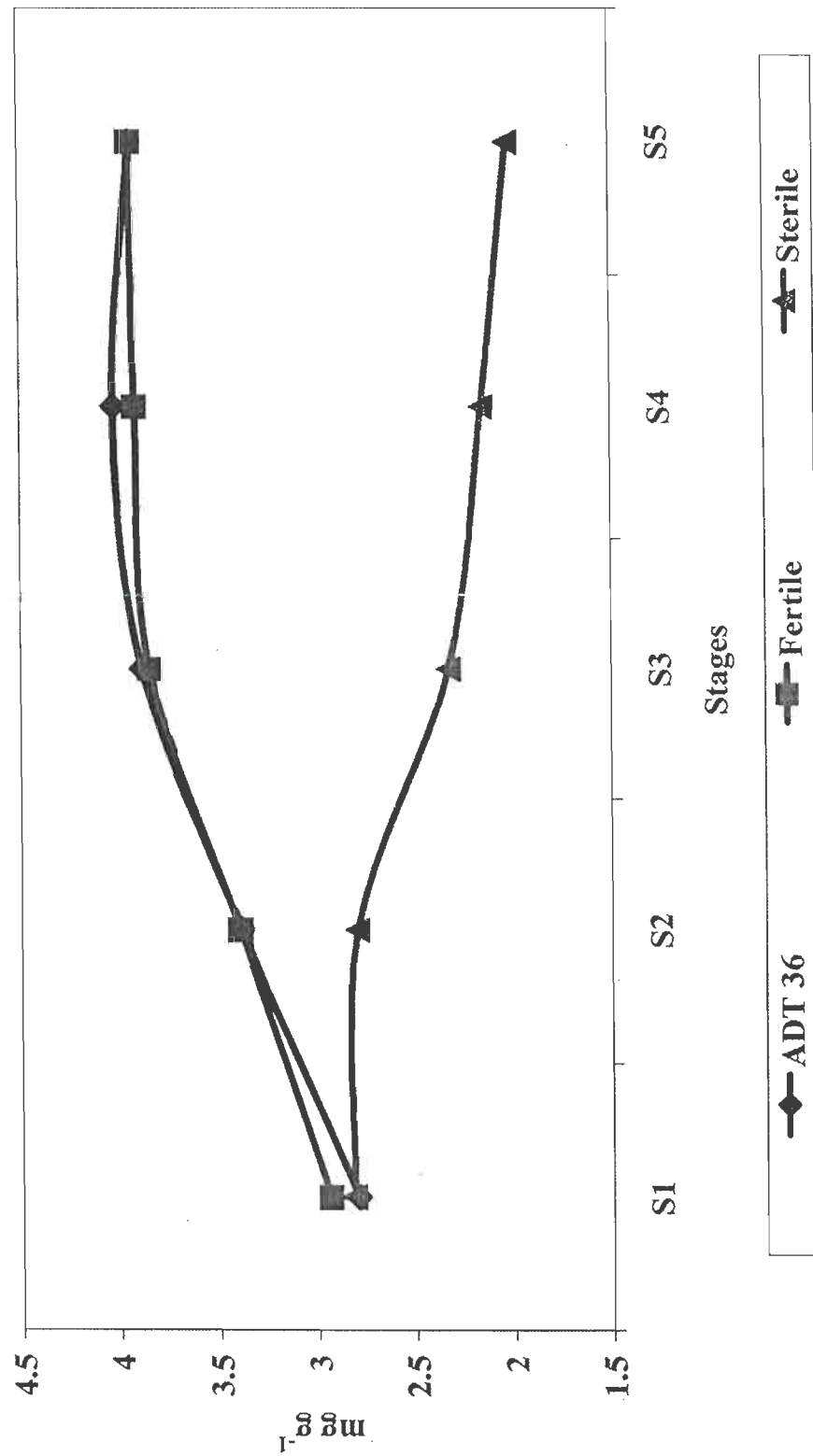
L S T L x S L x T S x T L x S x T
CD (P=0.05) 0.236 0.305 0.193 0.528 0.334 0.431 0.747

Table 19. Total free amino acid content (mg g^{-1}) in young panicles of TGMS lines under fertile and sterile conditions

TGMS lines	15 DBH				Flowering			
	Fertile	Sterile	% change		Fertile	Sterile	% change	
TGMS 6	0.98	0.67	32		1.72	1.02	41	
TGMS 16	1.01	0.72	29		1.64	0.97	41	
TGMS 29	1.06	0.72	33		1.78	1.13	37	
ADT 36	1.08	-	-		1.97	-	-	
Mean	1.01	0.70	31.3		1.71	1.04	39.6	

L S T L x S L x T S x T L x S x T
CD (P=0.05) 0.013 0.021 0.011 0.019 0.018 0.015 0.026

Fig.9 Total free amino acid content of TGMS lines under fertile and sterile conditions



49.3 per cent in stage 5 and from 31.3 per cent in 15 DBH to 39.6 per cent in flowering stage in young panicles. The varietal difference was minimum with TGMS 29 showing higher content under fertile condition values ranging from 3.14 mg g^{-1} to 4.09 mg g^{-1} in leaves (Fig. 9) and 1.06 mg g^{-1} in 15 DBH and 1.64 mg g^{-1} in flowering stage in young panicles. Amino acid accumulated continuously in fertile TGMS lines and ADT 36 in all the stages while in sterile lines it was decreasing. ADT 36 recorded the highest amino acid content in young panicles at 15 DBH (1.08 mg g^{-1}) and flowering stage (1.97 mg g^{-1}). The L, S, T, S x T and L x S x T interactions were significant from stage 2 onwards in leaves and all the interaction effects were significant in both the stages of young panicles.

4.3.2.5. Proline content

Proline content of TGMS lines under fertile and sterile conditions during different stages of panicle development in comparison with normal variety (ADT 36) is given in Tables 20 and 21. There was 3-5 fold accumulation of proline in leaves and 2-3 fold accumulation in young panicles under sterile condition in TGMS lines. Maximum accumulation was observed at stage 3 in leaves and at flowering stage in panicles under sterile condition with a mean value of 5.77 mg g^{-1} and 2.56 mg g^{-1} respectively. All the fertile TGMS lines and ADT 36 showed the same trend in proline accumulation over stages (Fig. 10). Among TGMS lines, TGMS 29 showed its supremacy in recording higher proline accumulation (5.92 mg g^{-1}) under sterile condition. The treatment and the interaction effects were significant from stage 2 onwards in leaves and in 15 DBH and flowering stages of young panicles.

4.3.2.6. Total phenol content

Data on total phenol content in Tables 22 and 23 show an increasing trend under sterile condition in all stages of panicle development in all TGMS lines. The increase was 17.3 per cent in stage 2 and 33.0 per cent in stage 5 in leaves (Fig. 11). In young panicles,

Table 20. Proline content (mg g^{-1}) in leaves of TGMS lines at different thermosensitive stages during fertility alteration

TGMS lines	S1			S2			S3			S4			S5		
	F	S	% change	F	S	% change	F	S	% change	F	S	% change	F	S	% change
TGMS 6	0.80	0.74	-	0.87	2.89	70	1.02	5.63	82	1.13	5.09	78	1.20	4.82	76
TGMS 16	0.85	0.81	-	0.94	3.05	70	1.15	5.76	81	1.23	5.19	77	1.23	4.94	76
TGMS 29	0.80	0.81	-	0.90	3.09	70	1.10	5.92	82	1.12	5.17	79	1.18	4.78	75
ADT 36	0.80	-	-	0.83	-	-	0.97	-	-	1.05	-	-	1.11	-	-
Mean	0.81	0.78	-	0.90	3.01	70.0	1.08	5.77	81.6	1.16	5.15	78.0	1.20	4.84	75.6

L S T L x S L x T S x T L x S x T
CD ($P=0.05$) 0.078 0.101 0.063 0.175 0.110 0.143 0.247

Table 21. Proline content (mg g^{-1}) in young panicles of TGMS lines under fertile and sterile conditions

TGMS lines	15 DBH			Flowering		
	Fertile	Sterile	% change	Fertile	Sterile	% change
TGMS 6	0.70	1.08	36	0.88	2.46	65
TGMS 16	0.88	1.34	35	1.03	3.12	67
TGMS 29	0.62	1.03	40	0.74	2.12	66
ADT 36	0.60	-	-	0.70	-	-
Mean	0.73	1.15	37.0	0.88	2.56	66.0

L S T L x S L x T S x T L x S x T
CD ($P=0.05$) 0.011 0.009 0.009 0.015 0.014 0.010 0.022

Fig.10 Proline content of TGMS lines under fertile and sterile conditions

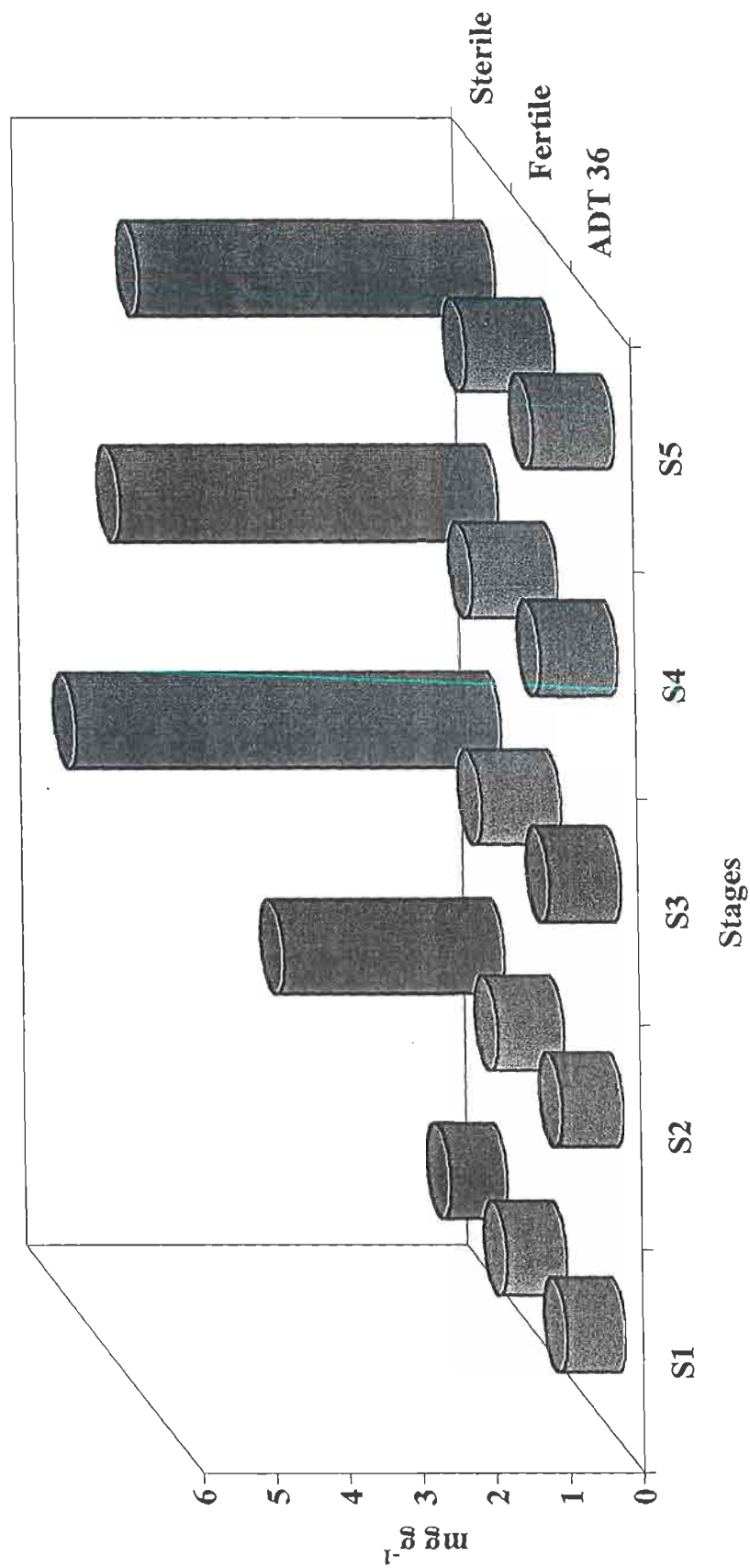


Table 22. Total phenol content (mg g^{-1}) in leaves of TGMS lines at different thermosensitive stages during fertility alteration

TGMS lines	S1			S2			S3			S4			S5		
	F	S	% change	F	S	% change	F	S	% change	F	S	% change	F	S	% change
TGMS 6	1.91	1.94	-	2.70	3.11	14	2.85	3.77	25	2.83	4.14	32	2.96	4.31	32
TGMS 16	2.08	2.12	-	2.82	3.36	17	2.92	3.90	26	2.95	4.04	39	2.85	4.29	34
TGMS 29	1.99	2.04	-	2.55	3.19	21	2.73	3.67	26	2.82	3.83	27	2.77	4.08	33
ADT 36	1.72	-	-	2.46	-	-	2.82	-	-	2.70	-	-	2.72	-	-
Mean	1.99	2.06	-	2.68	3.22	17.3	2.83	3.78	25.6	2.86	4.03	32.6	2.86	4.22	33.0

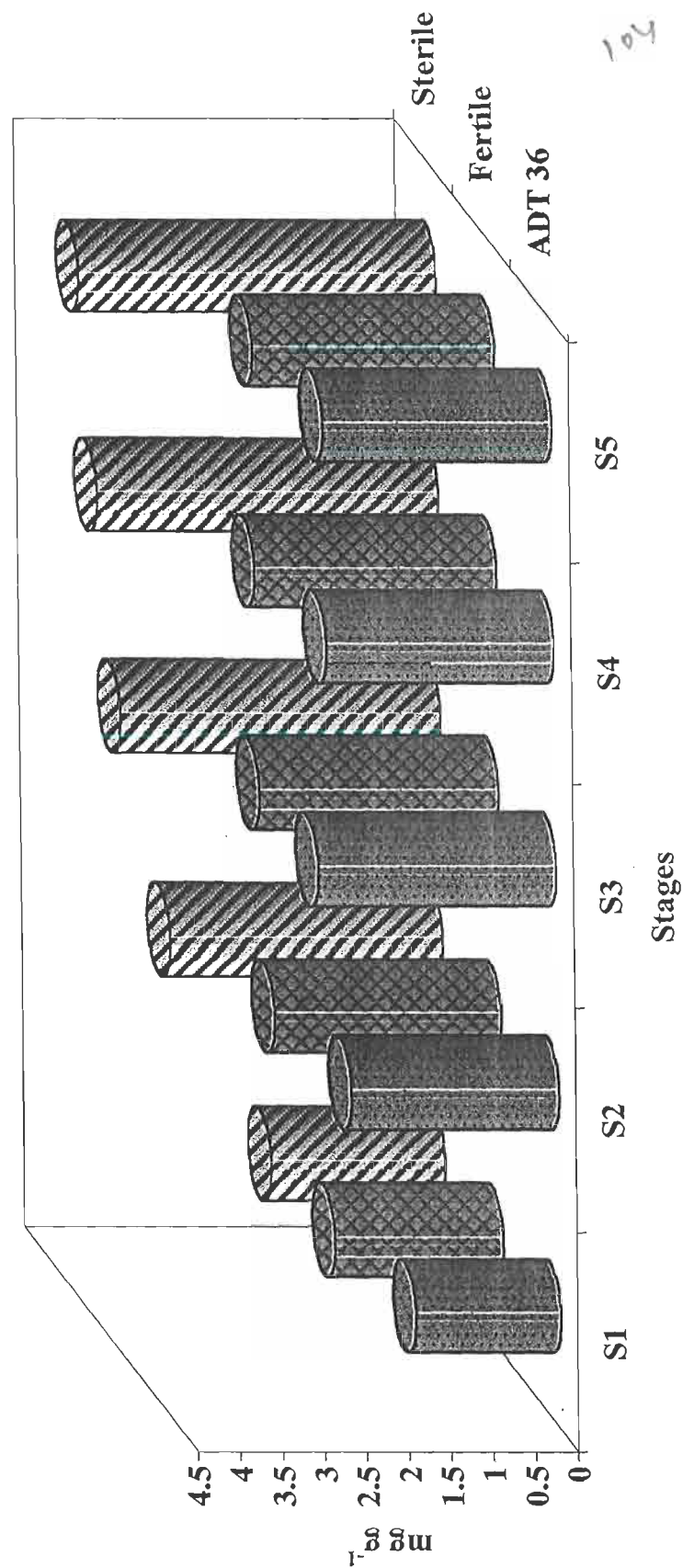
L S T L x S L x T S x T L x S x T
 CD ($P=0.05$) 0.227 0.292 0.185 0.507 0.320 0.414 0.717

Table 23. Total phenol content (mg g^{-1}) in young panicles of TGMS lines under fertile and sterile conditions

TGMS lines	15 DBH				Flowering			
	Fertile	Sterile	% change		Fertile	Sterile	% change	
TGMS 6	1.06	1.30	19		1.48	1.90	23	
TGMS 16	1.13	1.24	9		1.62	1.92	15	
TGMS 29	1.04	1.20	14		1.22	1.76	31	
ADT 36	1.03	-	-		1.18	-	-	
Mean	1.07	1.24	14.0		1.44	1.86	23.0	

L S T L x S L x T S x T L x S x T
 CD ($P=0.05$) 0.021 0.017 0.015 0.029 0.023 0.024 0.041

Fig.11 Total phenol content of TGMS lines under fertile and sterile conditions



the increase in total phenol was 14.0 per cent in 15 DBH and 23.0 per cent in flowering stage. Among the TGMS lines, TGMS 6 had higher phenol content in stage 5 (4.31 mg g^{-1}) in leaves and at flowering stage (19.0 mg g^{-1}) in young panicles under sterile condition. The fertile TGMS lines and ADT 36 performed similarly with no significant accumulation of phenols over stages. But there was significant difference observed between treatments in leaves and young panicles. The $S \times T$, $L \times S \times T$ interaction effects were highly significant in all the stages except stage 1 in leaves. The results suggested that there was accumulation of phenols in sterile condition even from stage 2, which showed its importance in fertility alteration.

4.3.2.7. Ascorbic acid content

The ascorbic acid content decreased significantly under sterile condition in all the TGMS lines at different stages of panicles development. Tables 24 and 25 show the ascorbic acid content of TGMS lines in comparison with normal variety (ADT 36) in leaves and panicles respectively. The ascorbic acid content reduced under sterile condition with a reduction from 24.0 per cent in stage 2 to 36.0 per cent in stage 5 in leaves of TGMS lines (Fig. 12). The young panicles showed significant reduction in 15 DBH (34.3%) and flowering stage (30.6%) under sterile condition (Table 24). ADT 36 recorded the highest value for ascorbic acid content under fertile condition in both the samples in all the stages. TGMS 16 showed higher percentage reduction in all the stages (29.0 to 40.0%) in leaves and TGMS 6 recorded higher percentage reduction at 15 DBH and flowering stage in young panicles (38.0 and 34.0%). The data showed significant treatment effect with minimal genotypic variation and significant interaction effects in all the stages except stage 1 in leaves.

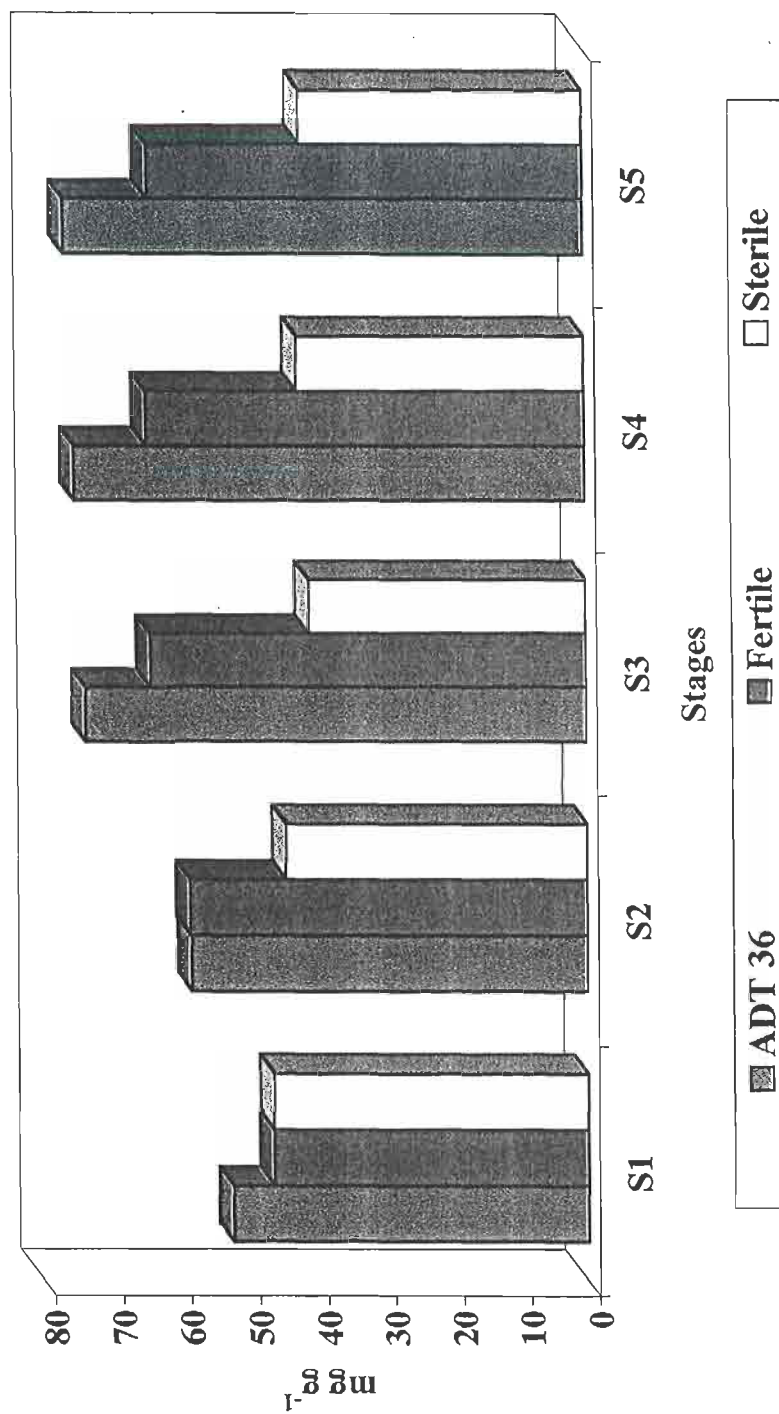
Table 24. Ascorbic acid content ($\mu\text{g g}^{-1}$) in leaves of TGMS lines at different thermosensitive stages during fertility alteration

TGMS lines	S1			S2			S3			S4			S5		
	F	S	% change	F	S	% change	F	S	% change	F	S	% change	F	S	% change
TGMS 6	44.00	43.92	-	57.75	43.95	25	64.07	40.65	38	64.27	42.42	35	63.52	41.80	35
TGMS 16	45.25	45.20	-	60.57	43.22	29	67.39	39.47	42	67.27	41.35	39	66.22	40.54	40
TGMS 29	49.45	49.65	-	56.75	46.00	18	60.75	42.27	30	61.82	43.37	30	62.30	42.87	33
ADT 36	52.23	-	-	58.17	-	-	73.62	-	-	75.08	-	-	76.32	-	-
Mean	46.22	46.25	-	58.35	44.39	24.0	64.07	40.79	36.6	64.45	42.38	34.6	64.01	41.73	36.0
L S T L x S L x T S x T L x S x T															
CD (P=0.05) 0.244 0.316 0.200 0.346 0.447 0.774															

Table 25. Ascorbic acid content ($\mu\text{g g}^{-1}$) in young panicles of TGMS lines under fertile and sterile conditions

TGMS lines	15 DBH				Flowering			
	Fertile	Sterile	% change		Fertile	Sterile	% change	
TGMS 6	32.10	20.47	38		48.41	32.21	34	
TGMS 16	28.16	18.70	36		43.80	29.17	33	
TGMS 29	28.44	20.08	29		40.72	30.77	25	
ADT 36	30.31	-	-		52.47	-	-	
Mean	29.56	19.75	34.3		44.31	30.77	30.66	
L S T L x S L x T S x T L x S x T								
CD (P=0.05) 0.417 0.342 0.340 0.589 0.579 0.481 0.834								

Fig.12 Ascorbic acid content of TGMS lines under fertile and sterile conditions



4.3.2.8.1. Deoxy ribonucleic acid (DNA) content

DNA content of leaves and young panicles of TGMS lines under fertile and sterile conditions at different stages was compared with normal variety (ADT 36) is shown in Tables 26 and 27. The DNA content of the TGMS lines was higher under fertile condition compared to sterile condition. The highest DNA content was recorded by TGMS 16 at stage 5 ($696.3 \mu\text{g g}^{-1}$) under fertile condition. In fertile TGMS lines, the DNA content increased significantly over stages with mean values ranged from $599.2 \mu\text{g g}^{-1}$ to $681.5 \mu\text{g g}^{-1}$ in leaves (Fig. 13) and from 308.6 mg g^{-1} to 469.0 mg g^{-1} in panicles. The percentage reduction of DNA content in sterile lines ranged from 14.3 in stage 2 to 35.61 in stage 5 in leaves and from 30.0 in 15 DBH to 37.0 in flowering stage in young panicles. The normal variety ADT 36 performed similar to that of fertile TGMS lines in both the samples in all the stages. Irrespective of the stages, significant differences were observed between lines and treatments. The interaction effects were also significant in all the stages in leaves and young panicles of TGMS lines.

4.3.2.8.2. Ribonucleic acid (RNA) content

Data on RNA content showed a decreasing trend under sterile condition in TGMS lines in all the stages of panicle development in leaves. The percentage reduction was 12.3, 27.3, 33.6 and 37.6 in stage 2, 3, 4 and 5 respectively over fertile lines (Table 28). But in young panicles, increasing trend was noticed in both the treatments, but the fertile panicles recorded significantly higher RNA content compared to sterile panicles in both the stages of sampling (Table 29). The leaf RNA content varied from $517.8 \mu\text{g g}^{-1}$ to $612.7 \mu\text{g g}^{-1}$ in fertile lines and $509.6 \mu\text{g g}^{-1}$ to $382.3 \mu\text{g g}^{-1}$ in sterile lines from stage 1 to stage 5 (Fig. 14). In young panicles, RNA content ranged from $341.3 \mu\text{g g}^{-1}$ in 15 DBH to $467.5 \mu\text{g g}^{-1}$ in flowering stage. The normal variety ADT 36 recorded the highest value for RNA content in leaves ($651.3 \mu\text{g g}^{-1}$) and young panicles (490.0 mg g^{-1}). The data showed

Table 26. Deoxy ribonucleic acid (DNA) content ($\mu\text{g g}^{-1}$) in leaves of TGMS lines at different thermosensitive stages during fertility alteration

TGMS lines	S1			S2			S3			S4			S5		
	F	S	% change	F	S	% change	F	S	% change	F	S	% change	F	S	% change
TGMS 6	585.5	503.1	15	655.5	518.4	21	640.4	493.3	23	668.3	446.0	34	668.8	440.8	35
TGMS 16	620.3	582.4	7	673.6	580.0	14	679.0	474.0	31	694.0	462.8	34	696.3	456.3	35
TGMS 29	606.7	585.7	4	643.0	586.4	8	657.4	493.6	25	673.0	436.1	36	682.1	432.7	37
ADT 36	643.0	-	-	660.8	-	-	671.5	-	-	678.6	-	-	680.3	-	-
Mean	599.2	556.6	8.6	657.7	561.3	14.3	661.7	486.6	26.3	678.2	448.0	34.6	681.5	442.6	35.6

L S T L x S L x T S x T L x S x T
 CD (P=0.05) 3.019 3.898 2.465 6.751 4.270 5.512 9.548

Table 27. Deoxy ribonucleic acid (DNA) content ($\mu\text{g g}^{-1}$) in young panicles of TGMS lines under fertile and sterile conditions

TGMS lines	15 DBH			Flowering		
	Fertile	Sterile	% change	Fertile	Sterile	% change
TGMS 6	287.0	196.0	32	408.0	287.0	30
TGMS 16	303.0	210.0	31	447.0	256.0	43
TGMS 29	336.0	248.0	27	503.0	312.0	38
ADT 36	362.0	-	-	518.0	-	-
Mean	308.6	218.0	30.0	469.0	285.3	37.0

L S T L x S L x T S x T L x S x T
 CD (P=0.05) 1.069 0.873 0.873 1.512 1.512 1.234 2.138

Fig.13 Deoxy ribonucleic acid (DNA) content of TGMS lines under fertile and sterile conditions

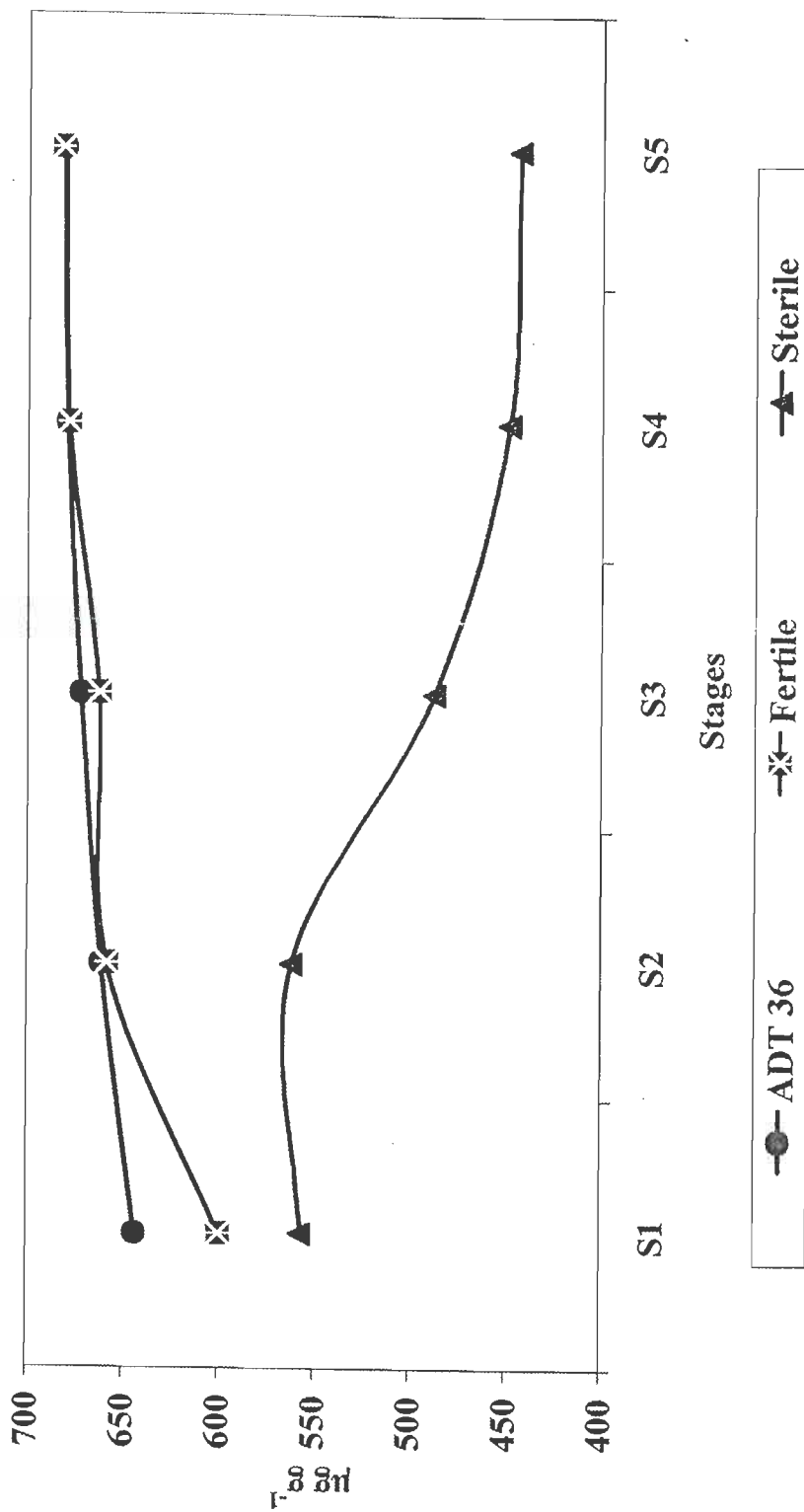


Table 28. Ribonucleic acid (RNA) content ($\mu\text{g g}^{-1}$) in leaves of TGMS lines at different thermosensitive stages during fertility alteration

TGMS lines	S1			S2			S3			S4			S5		
	F	S	% change	F	S	% change	F	S	% change	F	S	% change	F	S	% change
TGMS 6	522.0	512.6	-	550.6	491.3	11	575.8	432.9	25	594.8	405.2	32	602.1	384.8	37
TGMS 16	512.2	512.8	-	556.5	487.1	13	587.1	425.1	28	613.1	409.3	34	617.8	377.6	39
TGMS 29	506.9	505.1	-	545.5	478.1	13	581.9	416.4	29	603.6	392.6	35	611.5	386.0	37
ADT 36	528.1	-	-	568.6	-	-	598.3	-	-	623.1	-	-	651.3	-	-
Mean	517.8	509.6	-	548.5	485.3	12.3	577.7	424.3	27.3	599.0	403.3	33.6	612.7	382.3	37.6

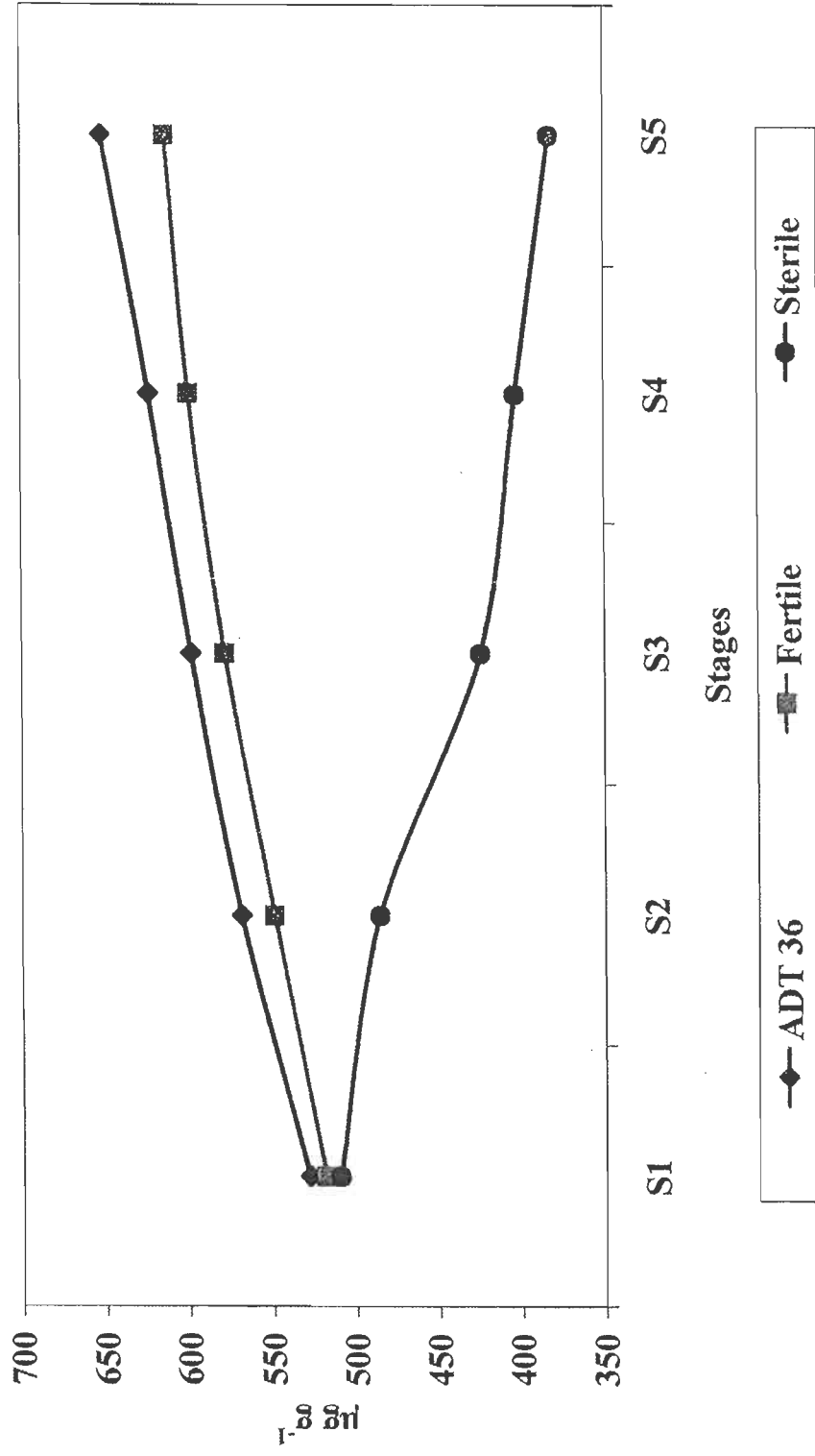
L S T L x S L x T S x T L x S x T
CD (P=0.05) 2.23 3.30 2.19 5.536 3.339 4.437 7.758

Table 29. Ribonucleic acid (RNA) content ($\mu\text{g g}^{-1}$) in young panicles of TGMS lines under fertile and sterile conditions

TGMS lines	15 DBH				Flowering			
	Fertile	Sterile	% change		Fertile	Sterile	% change	
TGMS 6	343.2	227.2	34		464.6	347.0	26	
TGMS 16	358.1	208.3	42		456.5	318.0	31	
TGMS 29	323.4	193.4	41		482.4	280.0	42	
ADT 36	364.0	-	-		490.0	-	-	
Mean	341.3	209.3	39.0		467.5	315.0	33.0	

L S T L x S L x T S x T L x S x T
CD (P=0.05) 5.132 4.191 4.101 7.259 7.208 5.927 10.265

Fig.14 Ribonucleic acid (RNA) content of TGMS lines under fertile and sterile conditions



a significant treatmental effect with minimal genotypic difference and with significant interaction effects in all the stages except stage 1 in leaves.

4.3.2.9. IAA Oxidase activity

IAA Oxidase activity of leaves and young panicles of TGMS lines under fertile and sterile condition in comparison with normal variety (ADT 36) during different panicle development stages is given in Tables 30 and 31. There was a significant treatment effect in terms of unoxidised auxin from stage 2 to 5 with per cent reduction being 15.33, 30.0, 35.3 and 35.3 respectively over fertile condition in leaves. And in panicles, it was 47.0 per cent on 15 DBH and 51.0 per cent at flowering stage. The mean values of unoxidised auxin in leaves of TGMS lines under fertile condition ranged from 15.37 to 16.38 μ moles $g^{-1} h^{-1}$ while it was from 15.39 to 10.65 μ moles $g^{-1} h^{-1}$ under sterile condition (Fig. 15). In young panicles the mean values of unoxidised auxin was 5.46 and 2.86 μ moles $g^{-1} h^{-1}$ in 15DBH and 8.25 and 4.08 μ moles $g^{-1} h^{-1}$ in flowering stage under fertile and sterile conditions respectively. Fertile TGMS lines and ADT 36 behaved similarly whereas all the TGMS lines followed the same trend. There were significant differences between the treatments and between the stages. The L x S, S x T and L x S x T interaction effects were significant in all the stages except stage 1 in leaves.

4.3.2.10. Nitrate reductase (NRase) activity

NRase activity of TGMS lines under fertile and sterile conditions in comparison with ADT 36 presented in Tables 32 and 33. Table 32 indicates the significant reduction in NRase activity under sterility with percentage reduction of 19.0, 42.3, 48.0 and 48.6 in stage 2, 3, 4 and 5 respectively in leaves. The NRase activity increased from 0.323 to 0.647 μ moles $NO_2 g^{-1} h^{-1}$ under fertile condition and from 0.324 to 0.333 μ moles $NO_2 g^{-1} h^{-1}$ under sterile condition from stage 1 to 5 (Fig. 16). Table 33 shows NRase

Table 30. IAA oxidase activity (μ moles of unoxidised auxin $\text{h}^{-1} \text{g}^{-1}$) in leaves of TGMS lines at different thermosensitive stages during fertility alteration

TGMS lines	S1			S2			S3			S4			S5		
	F	S	% change	F	S	% change	F	S	% change	F	S	% change	F	S	% change
TGMS 6	15.50	15.52	-	16.10	13.32	19	16.07	11.57	29	16.28	10.63	38	16.40	10.62	36
TGMS 16	15.77	15.85	-	16.60	14.75	13	16.92	11.77	31	16.74	11.12	34	16.87	10.82	36
TGMS 29	14.85	14.82	-	15.55	13.50	14	15.72	11.15	30	15.75	10.42	34	15.87	10.52	34
ADT 36	14.28	-	-	14.71	-	-	16.08	-	-	16.50	-	-	16.70	-	-
Mean	15.37	15.39	-	16.08	13.85	15.3	16.23	11.49	30.0	16.25	10.83	35.3	16.38	10.65	35.3

L S T L x S L x T S x T L x S x T
CD (P=0.05) 0.115 0.148 0.094 0.168 0.210 0.364

Table 31. IAA oxidase activity (μ moles of unoxidised auxin $\text{h}^{-1} \text{g}^{-1}$) in young panicles of TGMS lines under fertile and sterile conditions

TGMS lines	15 DBH				Flowering			
	Fertile	Sterile	% change		Fertile	Sterile	% change	
TGMS 6	5.42	2.78	49		8.12	3.88	53	
TGMS 16	5.68	2.92	48		8.60	4.26	51	
TGMS 29	5.30	2.90	44		8.04	4.10	49	
ADT 36	5.61	-	-		8.81	-	-	
Mean	5.46	2.86	47.0		8.25	4.08	51	

L S T L x S L x T S x T L x S x T
CD (P=0.05) 0.032 0.027 0.026 0.046 0.045 0.037 0.064

Fig.15 IAA oxidase activity of TGMS lines under fertile and sterile conditions

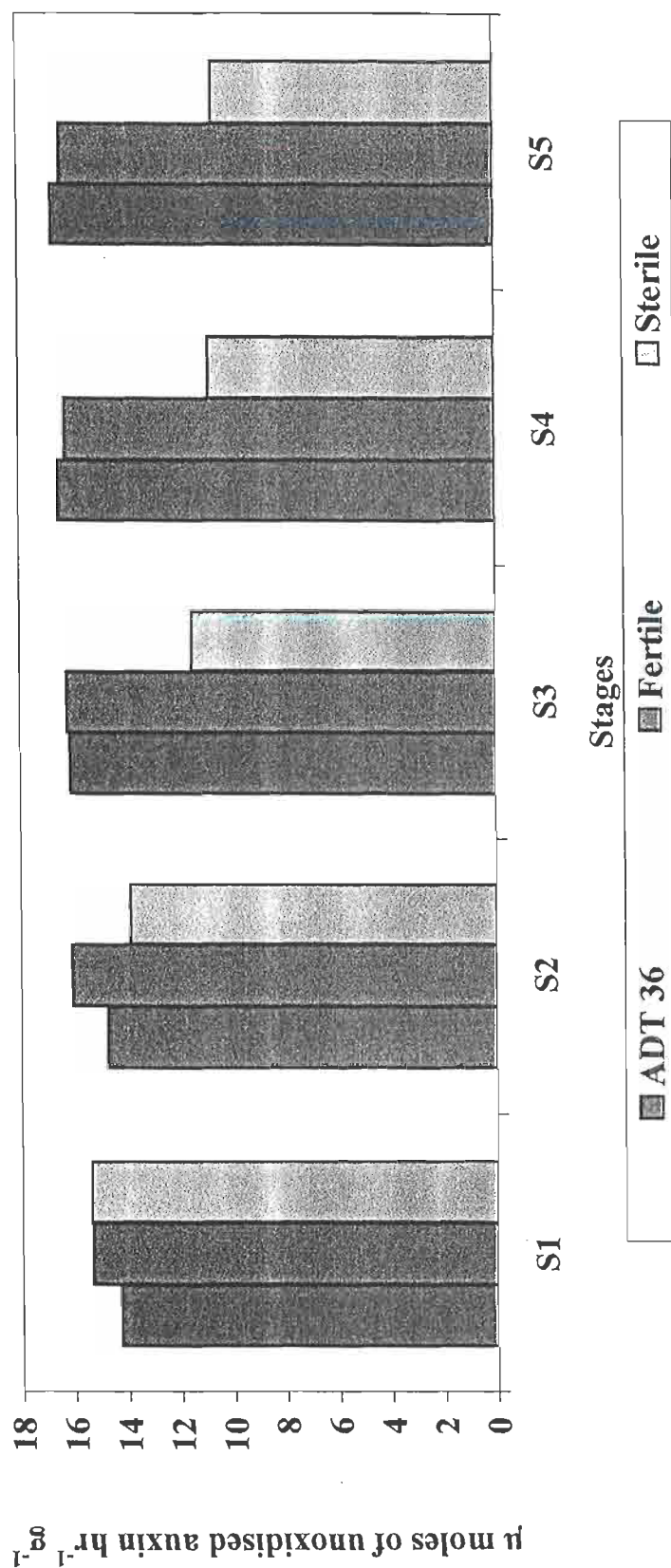


Table 32. NRase activity (μ moles of NO_2 produced $\text{g}^{-1} \text{h}^{-1}$) in leaves of TGMS lines at different thermosensitive stages during fertility alteration

TGMS lines	S1			S2			S3			S4			S5		
	F	S	% change	F	S	% change	F	S	% change	F	S	% change	F	S	% change
TGMS 6	0.298	0.301	-	0.387	0.310	20	0.548	0.312	44	0.618	0.318	49	0.633	0.331	48
TGMS 16	0.354	0.347	-	0.424	0.342	20	0.587	0.346	42	0.642	0.343	47	0.673	0.346	49
TGMS 29	0.318	0.324	-	0.392	0.327	17	0.542	0.322	41	0.621	0.325	48	0.635	0.324	49
ADT 36	0.358	-	-	0.390	-	-	0.590	-	-	0.654	-	-	0.668	-	-
Mean	0.323	0.324	-	0.401	0.326	19.0	0.559	0.326	42.3	0.627	0.328	48.0	0.647	0.333	48.6

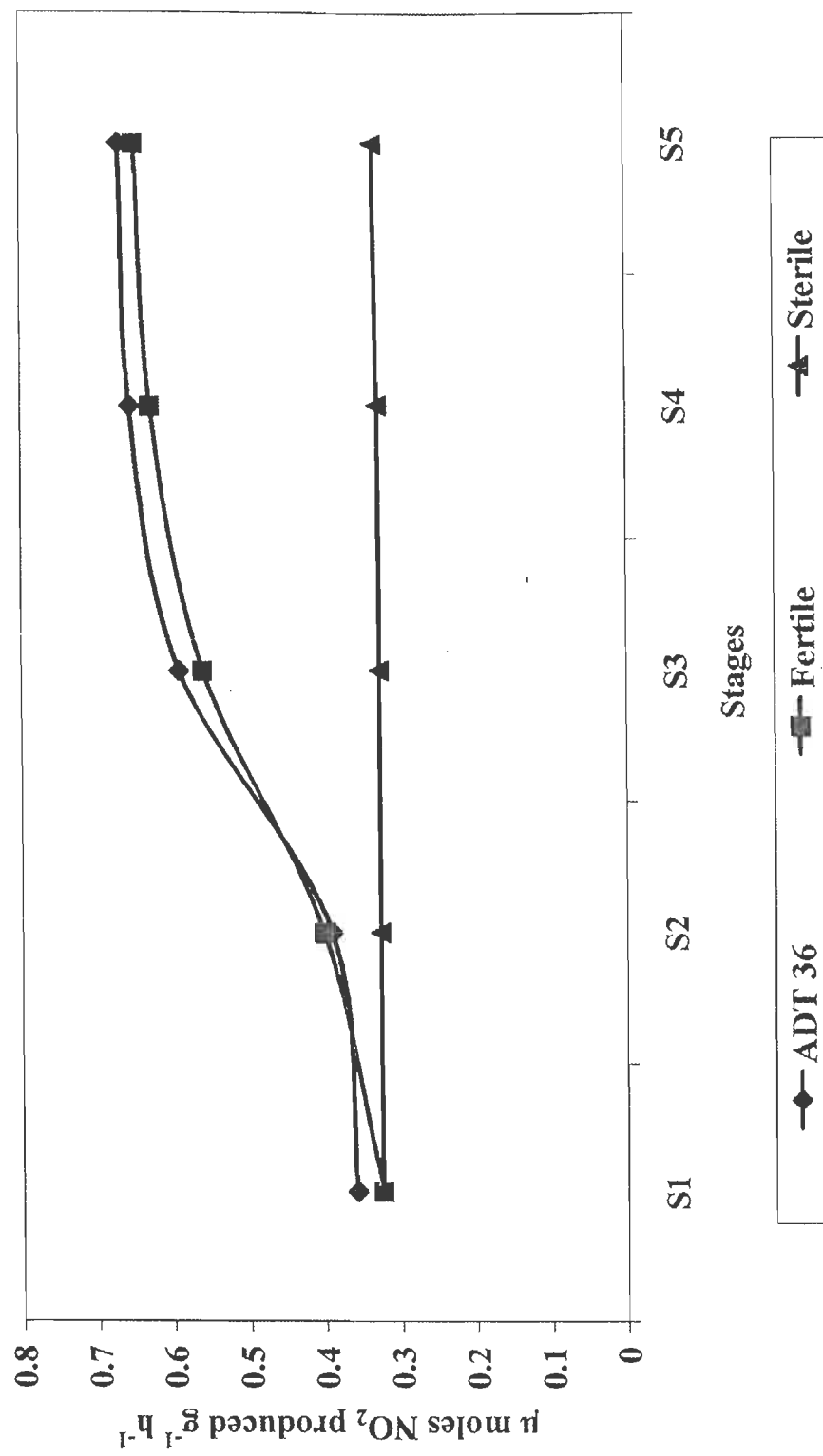
L S T L x S L x T S x T L x S x T
CD (P=0.05) 0.043 0.056 0.035 0.061 0.079 0.138

Table 33. NRase activity (μ moles of NO_2 produced $\text{g}^{-1} \text{h}^{-1}$) in young panicles of TGMS lines under fertile and sterile conditions

TGMS lines	15 DBH			Flowering		
	Fertile	Sterile	% change	Fertile	Sterile	% change
TGMS 6	0.143	0.096	33	0.212	0.122	43
TGMS 16	0.156	0.102	35	0.263	0.144	46
TGMS 29	0.160	0.102	37	0.243	0.128	48
ADT 36	0.168	-	-	0.251	-	-
Mean	0.153	0.100	35	0.239	0.131	45.6

L S T L x S L x T S x T L x S x T
CD (P=0.05) 0.004 0.003 0.003 0.005 0.005 0.008

Fig.16 NRase activity of TGMS lines under fertile and sterile conditions



activity of young panicles where the percentage reduction under sterile condition was 35.0 in 15 DBH and 45.6 in flowering stage.

Fertile TGMS lines and ADT 36 followed the same trend of increased activity over stages in both the samples. There was significant difference between treatments and between stages. The interaction effects (S x T and Lx S x T) were significant from stage 2 onwards.

4.3.2.11. Ascorbic acid oxidase (AAO) activity

Data on AAO activity showed an increasing trend under sterile condition at all the stages of panicle development in TGMS lines. There was 1.5 to 2.5 fold increase in the activity in leaves from stage 2 to stage 5 under sterile condition compared to fertile lines and 2 fold increase in young panicles at 15 DBH and flowering stage. The mean values of AAO activity in leaves of fertile and sterile TGMS lines from 2.58 to 2.54 enzyme units g^{-1} and 2.85 to 6.66 enzyme units g^{-1} from stage 1 to stage 5 respectively (Table 34 and Fig. 17). In young panicles, it was from 1.18 to 1.75 enzyme units g^{-1} and 2.10 to 3.13 enzyme units g^{-1} at 15 DBH and flowering stage respectively (Table 35). In leaves of fertile lines, the activity increased upto stage 3 and then decreased. ADT 36 followed the same trend as that of fertile lines. However, in sterile lines the activity increased steadily from stage 1 to stage 5 in leaves. Young panicles showed increasing trend in AAO activity in both the treatments. The difference between treatments, stages and the S x T interaction effects were significant in all the stages of sampling.

4.3.2.12. Glutamate dehydrogenase (GDH) activity

GDH activity of TGMS lines under fertile and sterile conditions in comparison with ADT 36 at various stages of panicle development is given in Tables 36 and 37. Highly significant variation was noticed between treatments in the activity of enzyme

Table 34. Ascorbic acid oxidase (AAO) activity (Enzyme units $\text{min}^{-1} \text{g}^{-1}$) in leaves of TGMS lines at different thermosensitive stages during fertility alteration

TGMS lines	S1			S2			S3			S4			S5		
	F	S	% change	F	S	% change	F	S	% change	F	S	% change	F	S	% change
TGMS 6	2.38	2.84	17	2.96	4.83	39	4.05	6.36	37	2.85	6.56	57	2.64	6.66	61
TGMS 16	2.65	2.93	10	3.00	4.96	40	3.19	6.40	51	2.74	6.68	59	2.54	6.75	63
TGMS 29	2.73	2.78	2	2.95	4.86	39	3.18	6.12	47	2.64	6.45	60	2.45	6.58	63
ADT 36	2.35	-	-	2.62	-	-	2.90	-	-	2.83	-	-	2.70	-	-
Mean	2.58	2.85	9.6	2.97	4.88	39.3	3.47	6.29	45.0	2.74	6.56	58.6	2.54	6.66	62.3
L S T L x S L x T S x T L x S x T CD (P=0.05) 0.128 0.165 0.164 0.287 0.181 0.234 0.405															

Table 35. Ascorbic acid oxidase (AAO) activity (Enzyme units $\text{min}^{-1} \text{g}^{-1}$) in young panicles of TGMS lines under fertile and sterile conditions

TGMS lines	15 DBH				Flowering			
	Fertile	Sterile	% change		Fertile	Sterile	% change	
TGMS 6	1.06	2.23	53		1.78	3.06	42	
TGMS 16	1.31	2.08	38		1.84	3.31	45	
TGMS 29	1.18	2.01	42		1.63	3.02	47	
ADT 36	1.05	-	-		1.58	-	-	
Mean	1.18	2.10	44.3		1.75	3.13	44.6	
L S T L x S L x T S x T L x S x T CD (P=0.05) 0.013 0.010 0.010 0.018 0.017 0.015 0.026								

Fig.17 Ascorbic acid oxidase (AAO) activity of TGMS lines under fertile and sterile conditions

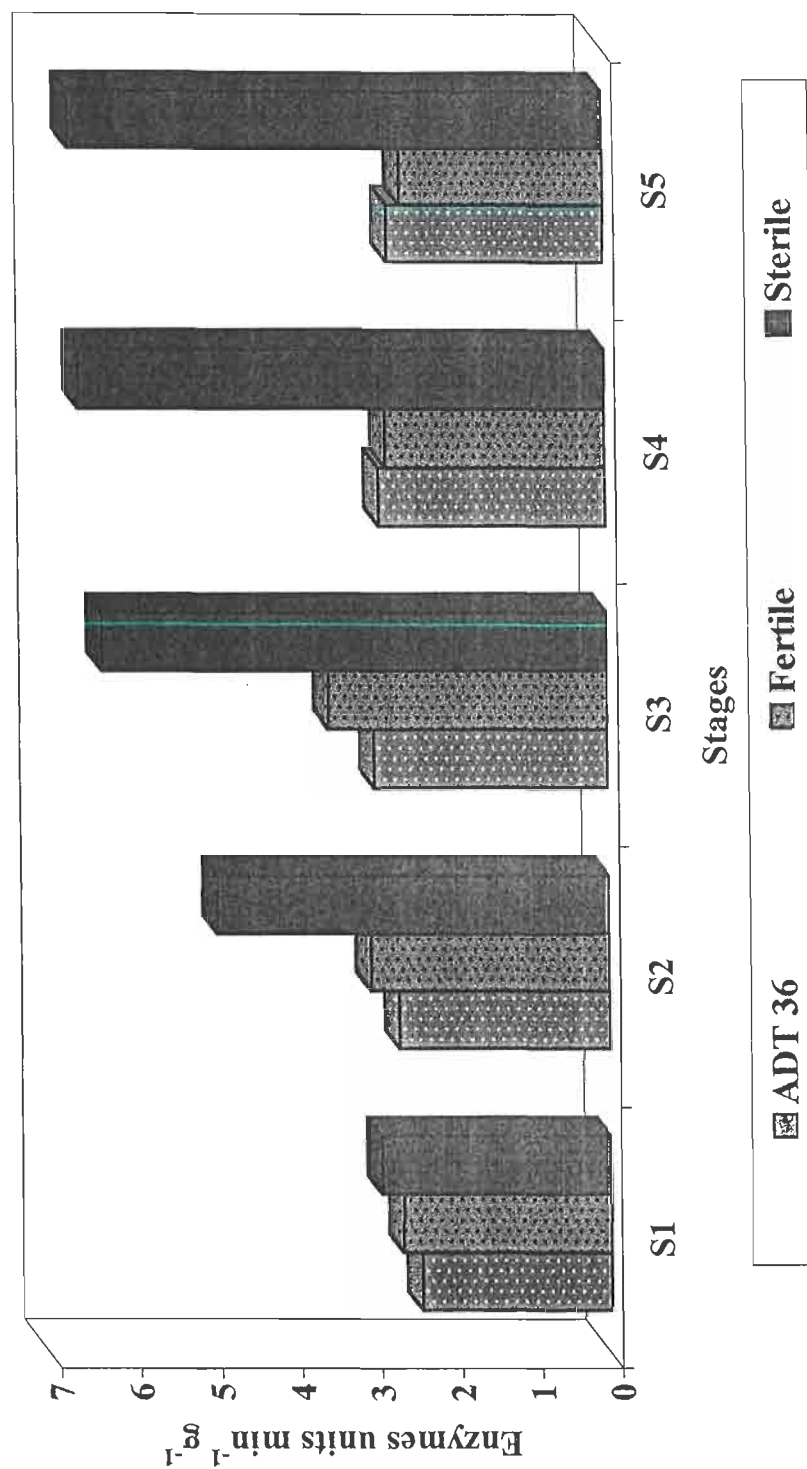


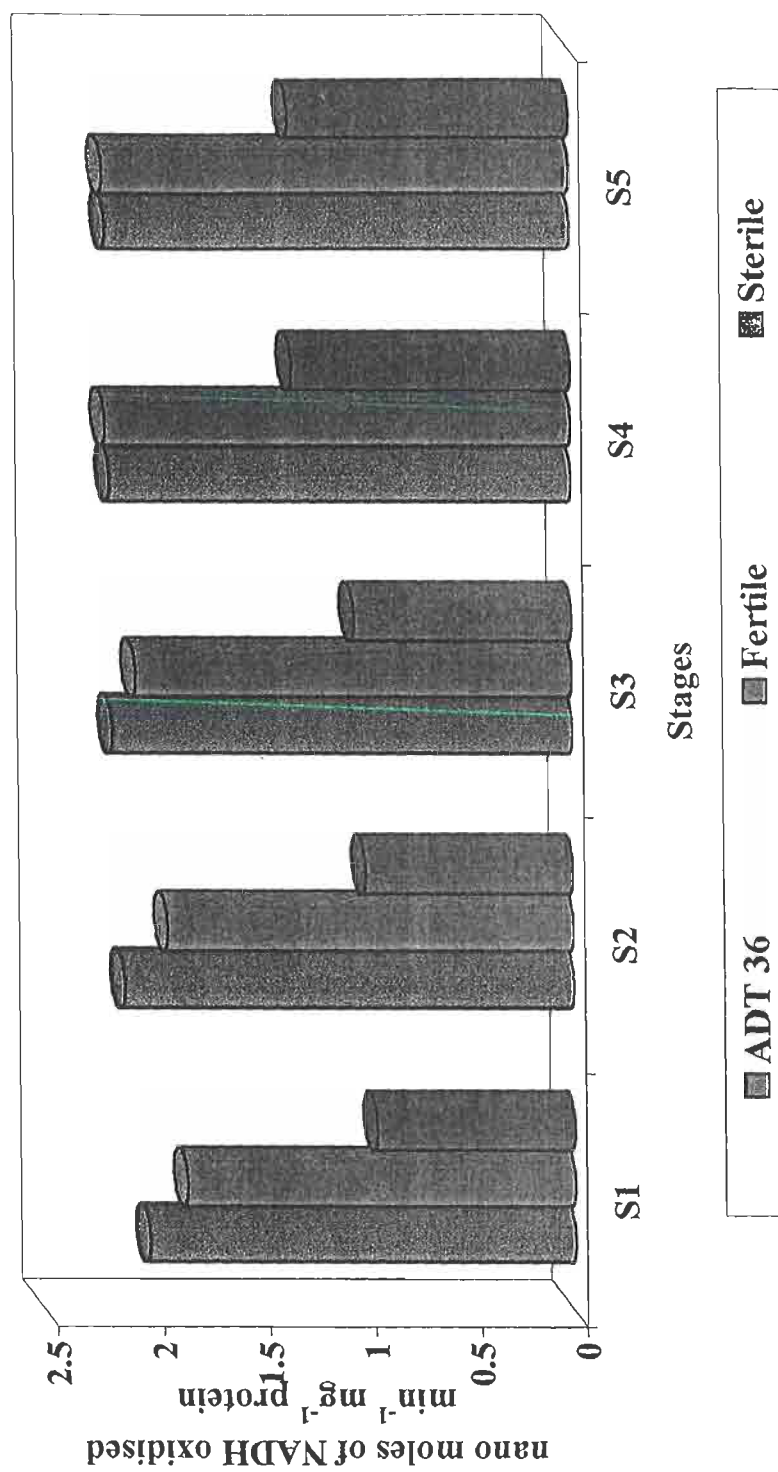
Table 36. Glutamate dehydrogenase activity (GDH) (nano moles of NADH oxidised min⁻¹ mg⁻¹ protein) in leaves of TGMS lines at different thermosensitive stages during fertility alteration

TGMS lines	S1			S2			S3			S4			S5		
	F	S	% change	F	S	% change	F	S	% change	F	S	% change	F	S	% change
TGMS 6	1.723	0.873	50	1.786	0.977	46	1.982	1.012	49	2.103	1.308	38	2.108	1.316	38
TGMS 16	1.816	0.992	46	1.946	0.996	49	2.086	1.046	50	2.336	1.318	44	2.314	1.327	43
TGMS 29	1.776	0.946	47	1.814	0.982	46	2.003	1.038	48	2.173	1.331	39	2.203	1.336	40
ADT 36	2.012	-	-	2.122	-	-	2.174	-	-	2.178	-	-	2.193	-	-
Mean	1.831	0.937	47.6	1.917	0.985	47.0	2.061	1.032	49.0	2.197	1.319	40.3	2.204	1.326	40.3
CD (P=0.05)															
				L		S	T	L x S	L x T	S x T	L x S x T				
				0.026		0.034	0.022	0.060	0.038	0.049	0.085				

Table 37. Glutamate dehydrogenase activity (GDH) (nano moles of NADH oxidised min⁻¹ mg⁻¹ protein) in leaves in young panicles of TGMS lines under fertile and sterile conditions

TGMS lines	15 DBH			Flowering		
	Fertile	Sterile	% change	Fertile	Sterile	% change
TGMS 6	0.846	0.322	62	1.166	0.573	51
TGMS 16	0.814	0.368	55	1.113	0.663	41
TGMS 29	0.855	0.311	64	1.204	0.518	51
ADT 36	0.852	-	-	1.286	-	-
Mean	0.838	0.333	60.3	1.161	0.484	49.6
CD (P=0.05)						
L		S	T	L x S	L x T	S x T
0.010		0.008	0.006	0.015	0.014	0.012
				L x S x T		
				0.021		

Fig.18 Glutamate dehydrogenase activity (GDH) of TGMS lines under fertile and sterile conditions



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GDH. The activity was reduced under sterile condition compared to fertile condition. The percentage reduction was 47.6, 47.0, 49.0, 40.3 and 40.3 from stage 1 to stage 5 respectively in leaves and 60.3 per cent at 15 DBH and 49.6% at flowering stage in young panicles. The mean values of GDH activity in leaves of fertile lines ranged from 1.831 to 2.204 nano moles of NADH oxidised $\text{min}^{-1} \text{mg}^{-1}$ protein from stage 1 to stage 5 and it was 0.838 and 1.161 nano moles NADH oxidised $\text{min}^{-1} \text{mg}^{-1}$ protein at 15 DBH (Fig. 18) and flowering stage respectively in young panicles. The variety ADT 36 behaved similarly as that of fertile TGMS lines in GDH activity. The S x T, L x T and L x S x T interactions were significant in all stages in both the samples.

4.3.2.13. Malate dehydrogenase (MDH) activity

Significant differences in MDH activity were exhibited by TGMS lines under fertile and sterile conditions at all thermosensitive stages. Table 38 indicates the MDH activity in leaves of TGMS lines in comparison with normal variety. There was a decreasing trend in MDH activity under sterile condition from 0.108 m moles of NADH oxidised $\text{min}^{-1} \text{ml}^{-1}$ of extract to 0.089 μ moles of NADH oxidised $\text{min}^{-1} \text{ml}^{-1}$ of extract (Fig. 19) and the percentage reduction was 28.3 to 32.6 from stage 1 to stage 5. But in young panicles, the MDH activity was 0.024 and 0.018 μ moles NADH oxidised $\text{min}^{-1} \text{ml}^{-1}$ extract at 15 DBH and 0.051 and 0.033 m moles NADH oxidised $\text{min}^{-1} \text{ml}^{-1}$ extract at flowering stage under fertile and sterile conditions respectively (Table 39). There was a significant difference between treatments and between stages. The interactions were also significant in all the stages except stage 1 in leaves.

4.3.2.14. Acid phosphatase (AP) activity

Acid phosphatase activity of leaves and young panicles of TGMS lines at different thermosensitive stages is given in Tables 40 and 41. The activity in leaves and panicles reduced significantly under sterile condition with percentage reduction of 25.0, 21.6, 22.0,

Table 38. Malate dehydrogenase activity (MDH) (μ moles of NADH oxidised $\text{min}^{-1} \text{ml}^{-1}$ of extract) in leaves of TGMS lines at different thermosensitive stages during fertility alteration

TGMS lines	S1			S2			S3			S4			S5		
	F	S	% change	F	S	% change	F	S	% change	F	S	% change	F	S	% change
TGMS 6	0.108	0.103	-	0.133	0.090	33	0.121	0.092	26	0.122	0.090	27	0.126	0.090	29
TGMS 16	0.123	0.114	-	0.125	0.093	26	0.128	0.096	25	0.132	0.093	30	0.134	0.091	33
TGMS 29	0.120	0.109	-	0.124	0.093	26	0.130	0.096	27	0.136	0.090	34	0.137	0.088	36
ADT 36	0.134	-	-	0.134	-	-	0.137	-	-	0.136	-	-	0.136	-	-
Mean	0.121	0.108	-	0.124	0.092	28.3	0.129	0.094	26.0	0.131	0.091	30.3	0.133	0.089	32.8

L S T L x S L x T S x T L x S x T
CD (P=0.05) 0.014 0.018 0.011 0.031 0.019 0.025 0.044

Table 39. Malate dehydrogenase activity (MDH) (μ moles of NADH oxidised $\text{min}^{-1} \text{ml}^{-1}$ of extract) in young panicles of TGMS lines under fertile and sterile conditions

TGMS lines	15 DBH				Flowering			
	Fertile	Sterile	% change		Fertile	Sterile	% change	
TGMS 6	0.022	0.017	33		0.045	0.033	27	
TGMS 16	0.026	0.017	35		0.053	0.038	29	
TGMS 29	0.026	0.020	24		0.050	0.029	42	
ADT 36	0.028	-	-		0.057	-	-	
Mean	0.024	0.018	30.6		0.051	0.033	32.6	

L S T L x S L x T S x T L x S x T
CD (P=0.05) 0.0014 0.0016 0.0011 0.0020 0.0020 0.0016 0.0028

Fig.19 Malate dehydrogenase activity (MDH) of TGMS lines under fertile and sterile conditions

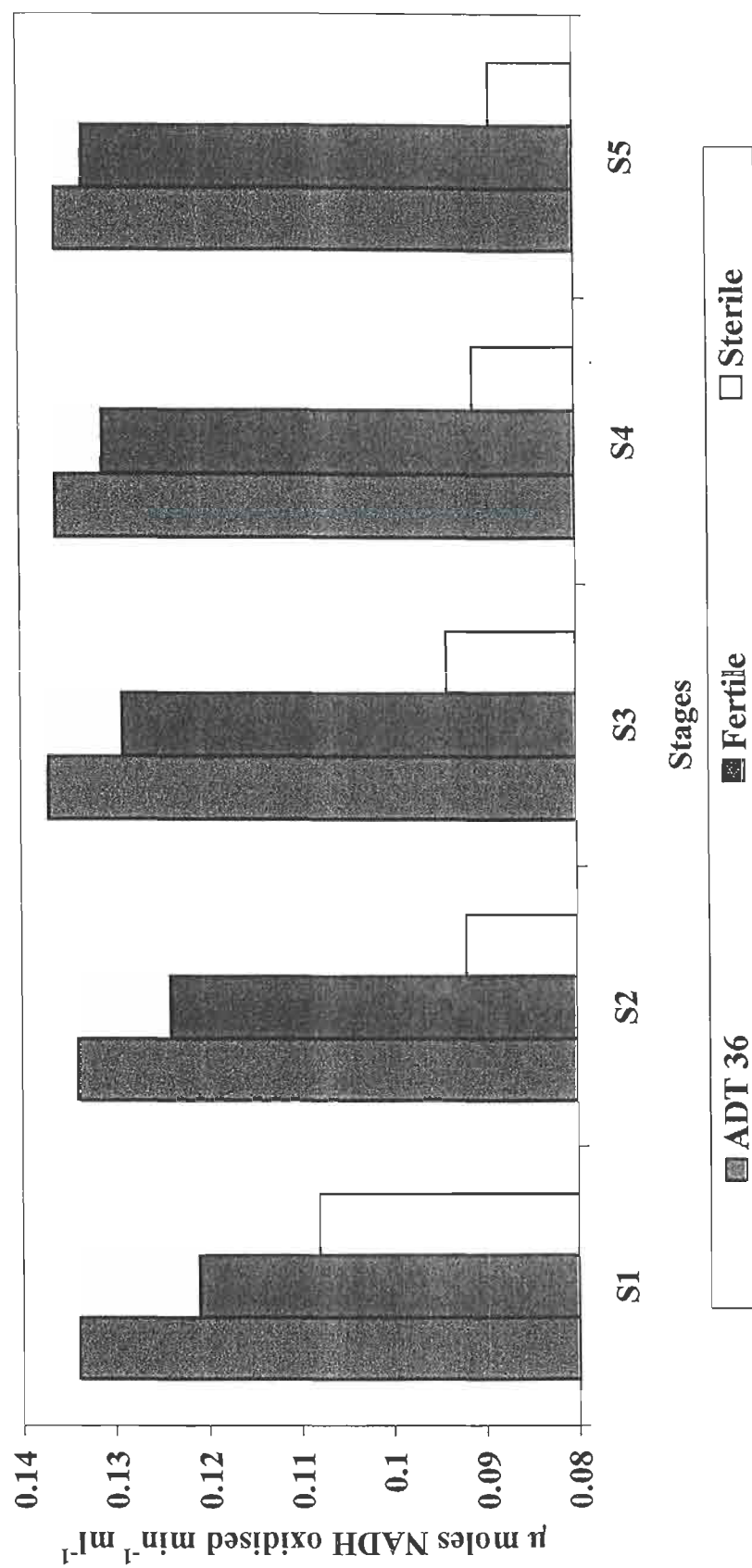


Table 40. Acid phosphatase activity (μ moles p-nitrophenol released $\text{min}^{-1} \text{mg}^{-1}$ protein) in leaves of TGMS lines at different thermosensitive stages during fertility alteration

TGMS lines	S1			S2			S3			S4			S5		
	F	S	% change	F	S	% change	F	S	% change	F	S	% change	F	S	% change
TGMS 6	582.7	423.1	28	596.3	464.1	23	627.8	482.9	24	681.5	508.4	26	687.3	509.7	26
TGMS 16	627.0	507.1	20	641.4	527.9	18	662.6	547.1	18	688.3	550.4	21	693.3	543.4	22
TGMS 29	662.3	482.3	28	681.2	520.4	24	688.7	523.8	24	697.1	528.1	25	697.8	530.1	24
ADT 36	587.3	-	-	602.3	-	-	641.8	-	-	663.7	-	-	677.4	-	-
Mean	614.6	470.6	25.3	630.0	503.6	21.6	654.5	517.3	22	682.2	528.6	24	688.5	527.3	24

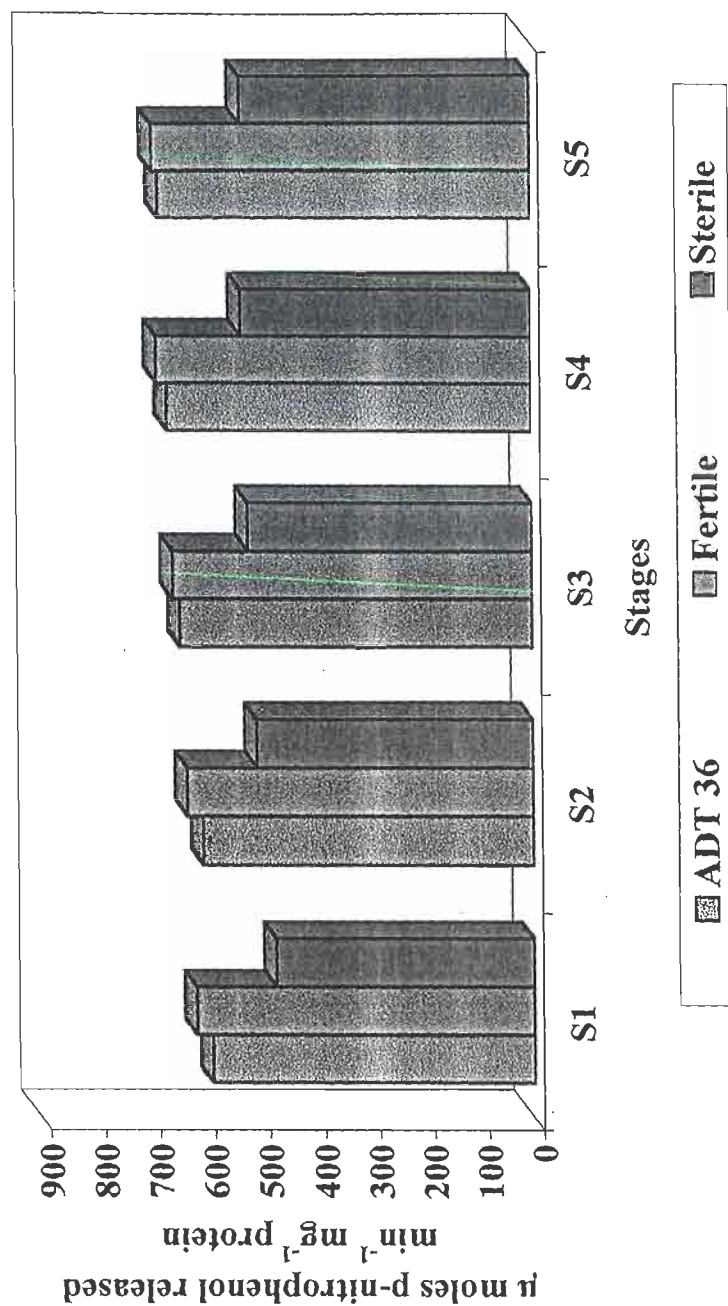
L S T L x S L x T S x T L x S x T
CD (P=0.05) 0.239 0.309 0.195 0.536 0.339 0.437 0.758

Table 41. Acid phosphatase activity (μ moles p-nitrophenol released $\text{min}^{-1} \text{mg}^{-1}$ protein) in young panicles of TGMS lines under fertile and sterile conditions

TGMS lines	15 DBH			Flowering		
	Fertile	Sterile	% change	Fertile	Sterile	% change
TGMS 6	162.4	130.0	20	433.3	276.2	37
TGMS 16	188.6	147.6	22	498.1	229.0	53
TGMS 29	170.4	132.4	23	468.7	267.7	43
ADT 36	203.1	-	-	494.3	-	-
Mean	173.8	136.6	21.6	466.3	257.3	45.0

L S T L x S L x T S x T L x S x T
CD (P=0.05) 0.814 0.664 0.664 0.152 0.152 0.9402 1.628

Fig.20 Acid phosphatase activity of TGMS lines under fertile and sterile conditions



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24.0 and 24.0 at stage 1, 2, 3, 4 and 5 respectively in leaves (Fig. 20) and 21.6 and 45.0 per cent at 15 DBH and flowering stage respectively in young panicles. The fertile lines and ADT 36 had the same trend of increased acid phosphatase activity over stages in both the samples whereas in sterile lines there was a reduction in activity when compared to fertile ones. The highest acid phosphatase activity (697.8 μ moles p-nitrophenol released $\text{min}^{-1} \text{mg}^{-1}$ protein) was noticed in leaves of TGMS 29 at stage 5 under fertile condition. The treatments, stages and the interaction effects were significant at all stages in both the samples.

4.3.2.15. Catalase activity

The catalase activity in leaves of TGMS lines under fertile and sterile conditions during fertility transformation stages in comparison with normal variety (ADT 36) is presented in Table 42. The activity decreased significantly under sterile condition accounting for 41.3 per cent to 65.0 per cent from stage 2 to stage 5 compared to fertility. The mean values of activity ranged from 2.18 to 3.63 enzyme units $\times 10^4 \text{ g}^{-1} \text{ min}^{-1}$ in fertile lines and from 2.15 to 1.29 enzyme units $\times 10^4 \text{ g}^{-1} \text{ min}^{-1}$ in sterile lines from stage 1 to stage 5. The catalase activity increased steadily in fertile lines and in ADT 36, but reduced continuously over stages in sterile lines (Fig. 21). The variety ADT 36 recorded the maximum enzyme activity (3.75 enzyme units $\times 10^4 \text{ g}^{-1} \text{ min}^{-1}$) at stage 4 and TGMS 29 at stage 3 recorded the minimum value (1.21 enzyme units $\times 10^4 \text{ g}^{-1} \text{ min}^{-1}$) under sterile condition. Table 43 shows the catalase activity of young panicles under fertile and sterile conditions. The fertile TGMS lines recorded higher values at 15 DBH (1.19 enzyme units $\times 10^4 \text{ g}^{-1} \text{ min}^{-1}$) and at flowering stage (2.04 enzyme units $\times 10^4 \text{ g}^{-1} \text{ min}^{-1}$) over sterile lines. There was a significant difference between treatments with minimum variation between genotypes and significant S $\times$ T and L $\times$ S $\times$ T interaction effects from stage 2 onwards in leaves.

Table 42. Catalase activity (Enzyme units $\times 10^4 \text{ g}^{-1} \text{ min}^{-1}$) in leaves of TGMS lines at different thermosensitive stages during fertility alteration

TGMS lines	S1			S2			S3			S4			S5		
	F	S	% change	F	S	% change	F	S	% change	F	S	% change	F	S	% change
TGMS 6	2.28	2.26	-	2.71	1.61	41	3.33	1.26	70	3.69	1.30	65	3.64	1.25	66
TGMS 16	2.16	2.13	-	2.62	1.57	41	3.31	1.32	61	3.73	1.49	61	3.71	1.32	65
TGMS 29	2.16	2.08	-	2.73	1.60	42	3.25	1.21	63	3.61	1.25	66	3.55	1.31	64
ADT 36	2.20	-	-	2.88	-	-	3.50	-	-	3.88	-	-	3.75	-	-
Mean	2.18	2.15	-	2.68	1.59	41.3	3.29	1.26	64.6	3.67	1.34	64.0	3.63	1.29	65.0

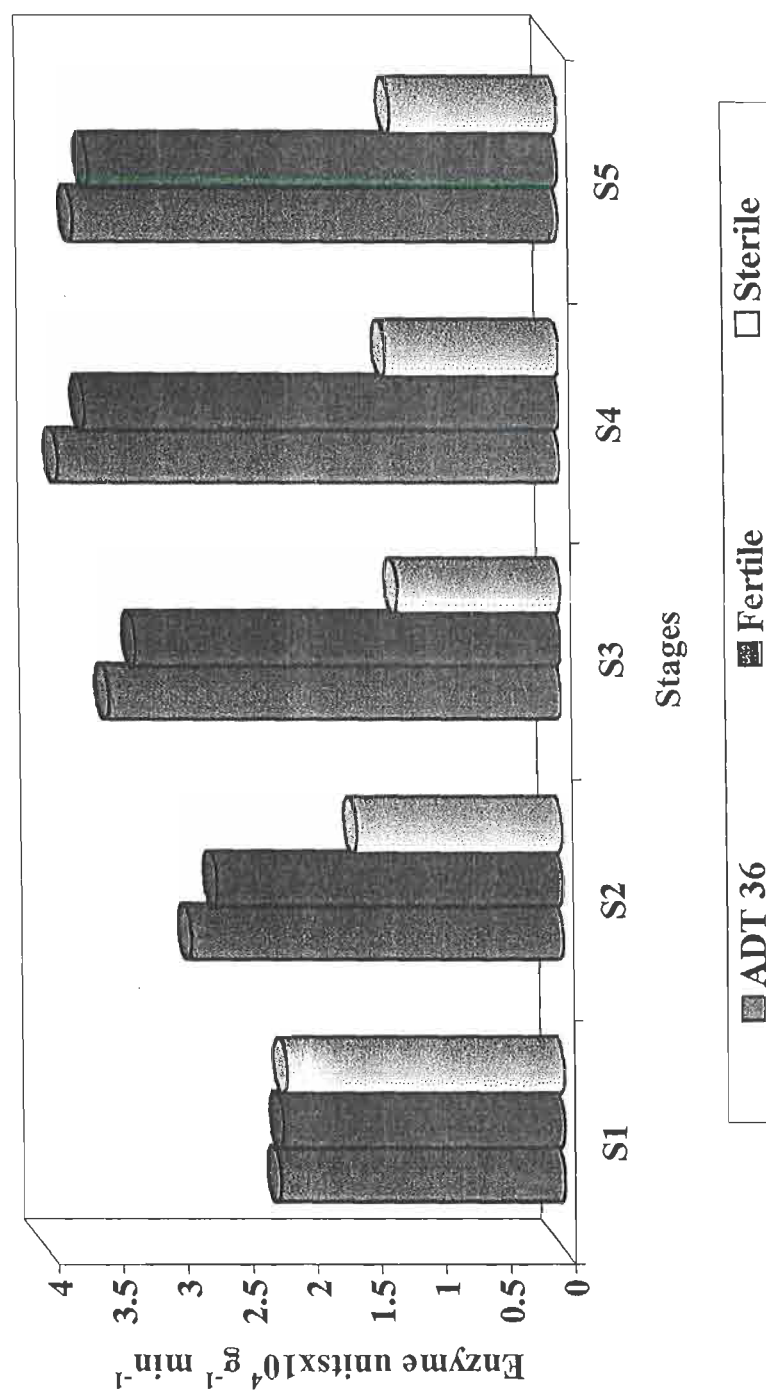
L S T L x S L x T S x T L x S x T
 CD (P=0.05) 0.079 0.102 0.064 0.177 0.112 0.144 0.250

Table 43. Catalase activity (Enzyme units $\times 10^4 \text{ g}^{-1} \text{ min}^{-1}$) in young panicles of TGMS lines under fertile and sterile conditions

TGMS lines	15 DBH			Flowering		
	Fertile	Sterile	% change	Fertile	Sterile	% change
TGMS 6	1.23	0.96	22	2.08	1.16	45
TGMS 16	1.20	0.84	30	2.21	1.03	54
TGMS 29	1.14	0.80	30	1.83	0.93	50
ADT 36	1.15	-	-	2.80	-	-
Mean	1.19	0.86	27.3	2.04	1.04	49.6

L S T L x S L x T S x T L x S x T
 CD (P=0.05) 0.027 0.023 0.022 0.039 0.038 0.031 0.055

Fig.21 Catalase activity of TGMS lines under fertile and sterile conditions



4.3.2.16. Peroxidase activity

Peroxidase activity of leaves and young panicles of TGMS lines under fertile and sterile conditions in comparison with normal variety (ADT 36) at various stages of thermosensitivity is given in Tables 44 and 45. The activity in leaves reduced continuously over stages with a per cent reduction of 18.0 from stage 2 to 37.6 in stage 5 (Table 44). The activity reduced from 172.2 to 114.4 enzyme units $l^{-1} h^{-1}$ under sterile condition but remained steady under fertile condition from stage 1 to stage 5 (173.9 to 182.1 enzyme units $l^{-1} h^{-1}$). ADT 36 had a gradual increase in activity from stage 1 to stage 5 and recorded maximum value for peroxidase activity at stage 5 (197.4 enzyme unit $l^{-1} h^{-1}$) in fertile lines (Fig. 22). In young panicles, the activity was high under fertile condition compared to sterile condition in all the TGMS lines and in ADT 36 (Table 45). The percentage reduction in activity was 32.3 at 15 DBH and 23.6 at flowering stage under sterile condition. Treatmental effects and the $S \times T$ and $L \times S \times T$ interaction effects were significant at all stages except stage 1 in leaves.

4.3.2.17. Superoxide distmutase (SOD) activity

SOD activity reduced significantly under sterile condition in TGMS lines compared to fertile lines in both leaves and panicles. The activity in leaves reduced from 7.82 to 5.29 enzyme units mg^{-1} protein h^{-1} in sterile lines from stage 1 to stage 5 (Table 46). The activity increased slowly and continuously in fertile lines and in ADT 36 whereas a steady reduction was noticed in sterile TGMS lines (Fig. 23). The highest activity was noticed in fertile TGMS 29 (8.47 enzyme units mg^{-1} protein h^{-1}) at stage 5 and the lowest was recorded in sterile TGMS 6 at stage 3 (5.08 enzyme units mg^{-1} protein h^{-1}). Table 47 shows the SOD activity of young panicles at 15 DBH and at flowering stage. There was a significant treatmental effect in both the stages with per cent reduction being 37.3 at 15 DBH and 60.3 at flowering stage over fertile condition. The treatment effect was

Table 44. Peroxidase activity (Enzyme units $l^{-1} hr^{-1}$) in leaves of TGMS lines at different thermosensitive stages during fertility a¹teration

TGMS lines	S1			S2			S3			S4			S5		
	F	S	% change	F	S	% change	F	S	% change	F	S	% change	F	S	% change
TGMS 6	175.2	176.0	-	178.5	153.2	15	184.7	123.0	34	185.5	125.0	33	190.5	119.5	38
TGMS 16	170.7	166.0	-	180.5	144.7	20	185.5	116.0	38	182.7	113.5	38	179.7	119.2	34
TGMS 29	176.0	174.7	-	177.2	144.0	19	181.2	111.2	39	179.5	102.2	44	176.2	104.7	41
ADT 36	170.2	-	-	181.6	-	-	190.7	-	-	192.3	-	-	197.4	-	-
Mean	173.9 6	172.2 3	-	178.8	147.3	18.0	183.8	116.3	37.0	182.5	113.5	38.0	182.3	114.4	37.6

L S T L x S L x T S x T L x S x T
CD (P=0.05) 0.079 0.102 0.064 0.177 0.112 0.144 0.250

Table 45. Peroxidase activity (Enzyme units $l^{-1} hr^{-1}$) in young panicles of TGMS lines under fertile and sterile conditions

TGMS lines	15 DBH			Flowering		
	Fertile	Sterile	% change	Fertile	Sterile	% change
TGMS 6	52.0	34.0	35	90.0	67.0	26
TGMS 16	56.0	38.0	33	98.0	72.0	27
TGMS 29	59.0	42.0	29	106.0	87.0	18
ADT 36	55.0	-	-	93.0	-	-
Mean	55.66	38.0	32.3	98.0	75.3	23.6

L S T L x S L x T S x T L x S x T
CD (P=0.05) 0.247 0.203 0.202 0.350 0.286 0.348 0.495

Fig.22 Peroxidase activity of TGMS lines under fertile and sterile conditions

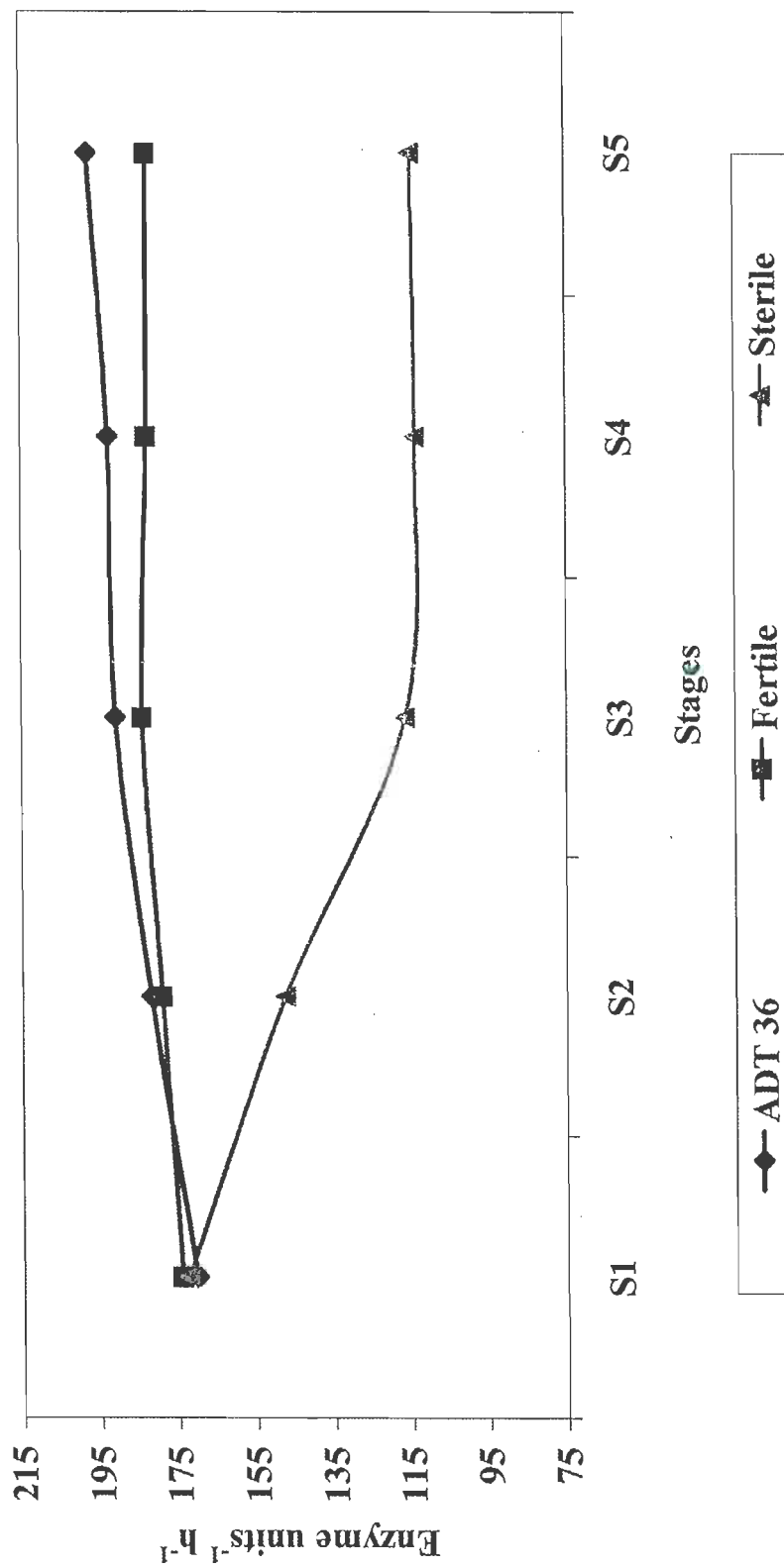


Table 46. Superoxide dismutase (SOD) activity (Enzyme units mg^{-1} protein hr^{-1}) in leaves of TGMS lines at different thermosensitive stages during fertility alteration

TGMS lines	S1			S2			S3			S4			S5		
	F	S	% change	F	S	% change	F	S	% change	F	S	% change	F	S	% change
TGMS 6	7.63	7.66	-	7.85	6.22	21.0	7.99	5.08	37.0	8.12	5.16	37.0	8.19	5.28	36.0
TGMS 16	7.82	7.80	-	8.03	6.41	20.0	8.19	5.30	35.0	8.30	5.39	36.0	8.38	5.32	37.0
TGMS 29	8.08	8.00	-	8.28	6.36	24.0	8.35	5.33	37.0	8.41	5.30	37.0	8.47	5.28	38.0
ADT 36	7.33	-	-	7.52	-	-	7.73	-	-	7.92	-	-	8.17	-	-
Mean	7.84	7.82	-	8.05	6.33	21.6	8.17	5.23	36.3	8.27	5.28	36.6	8.34	5.29	37.0

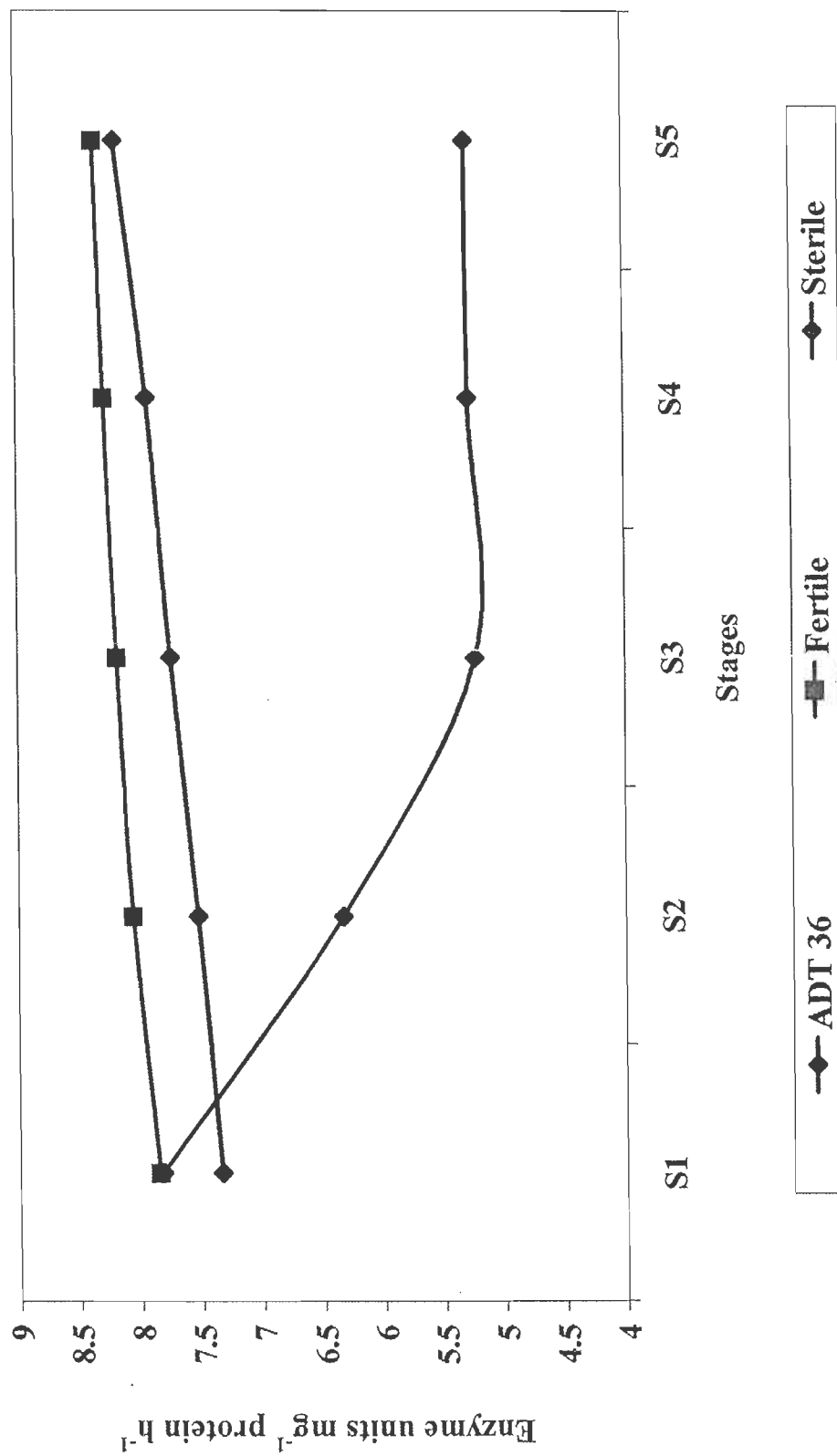
L S T L x S L x T S x T L x S x T
 CD (P=0.05) 0.318 0.362 0.287 0.778 0.502 0.618 1.072

Table 47. Superoxide dismutase (SOD) activity (Enzyme units mg^{-1} protein hr^{-1}) in young panicles of TGMS lines under fertile and sterile conditions

TGMS lines	15 DBH				Flowering			
	Fertile	Sterile	% change		Fertile	Sterile	% change	
TGMS 6	1.66	1.02	39		3.41	1.32	62	
TGMS 16	1.58	0.98	38		3.62	1.50	59	
TGMS 29	1.61	1.05	35		3.37	1.35	60	
ADT 36	1.74	-	-		4.08	-	-	
Mean	1.61	1.61	37.33		3.62	1.39	60.3	

L S T L x S L x T S x T L x S x T
 CD (P=0.05) 0.013 0.011 0.010 0.019 0.018 0.015 0.026

Fig.23 Superoxide dismutase (SOD) activity of TGMS lines under fertile and sterile conditions



significant with minimum genotypic variation and the interaction effects were also significant in all the stages of sampling except the stage 1 in leaves.

4.3.3. Molecular mechanism of TGMS

4.3.3.1. Study of protein profiles in TGMS lines under sterile and fertile condition

The SDS-PAGE profile of protein extracts from the lines TGMS 6, TGMS 16 and TGMS 29 under fertile and sterile conditions revealed that, there were detectable differences between fertile and sterile conditions in leaves (Plate 3) and anthers (Plate 4). Though many minor variations were observed in protein profiles for leaves and anthers prominent detectable variations are taken into consideration. The molecular weight of the bands are approximated based on R_m values. The variation in protein profiles observed is given in the Table 48.

From the Table 48, it was evident that, there was little qualitative change observed in protein profiles between fertile and sterile conditions. But the samples showed distinct quantitative differences under fertile and sterile conditions. There was a reduction in expression of 64, 54 and 24 kDa protein in leaves and 48, 38 and 24 kDa proteins in anthers under sterile condition. But a small molecular weight protein (14 kDa) was present in sterile TGMS lines and was totally absent in fertile lines. The reduction in the expression of larger subunit of Rubisco (50-55 kDa) was distinct in sterile plants. A high molecular weight protein (64 kDa) and a low molecular weight protein (24 kDa) also showed a different expression pattern in terms of quantity under fertile and sterile conditions in TGMS lines, so it was inferred that the content of the above said molecular weight proteins was reduced under sterile condition.

Table 48. SDS-PAGE protein profile study of fertile and sterile TGMS lines

Lines	Molecular weight (kDa)	Leaves		Molecular weight (kDa)	Anthers	
		Fertile	Sterile		Fertile	Sterile
TGMS 6	64	+++	+	48	+++	+
	54	+++	+	38	+++	+
	24	+++	+	24	+++	+
				14	+	+++
TGMS 16	64	+++	+	48	+++	+
	54	+++	+	38	+++	+
	24	+++	+	24	+++	+
				14	+	+++
TGMS 29	64	+++	+	48	+++	+
	54	+++	+	38	+++	+
	24	+++	+	24	+++	+
				14	+	+++

Legend : +++ : High intensity
 + : Low intensity

4.3.3.2. Isozyme analysis

4.3.3.2.1. Free radical scavenging enzymes

4.3.3.2.1.1. Catalase (CAT)

Isoenzymatic pattern of catalase enzyme is depicted in Plate 5. The availability of various isoforms in selected TGMS lines under fertile and sterile conditions are shown in Table 49. From this, it was observed that the fertile TGMS lines exhibited 3 isoforms *viz.*, CAT 1, 2 and 3 as the maximum number. Under sterile condition it produced 2 (TGMS 29) or 1 (TGMS 6 and 16) isoforms. The intensity of isoforms were also high under fertile condition. Isoform 3rd had shown that its presence was highly correlated with fertility.

4.3.3.2.2. Peroxidase (POX)

The zymogram of peroxidase is represented in the Table 49 based on isozyme pattern in the Plate 6. In the banding sequence TGMS 6, TGMS 16 and TGMS 29 under fertile condition produced 6, 6 and 5 bands respectively, whereas sterile TGMS 6, 16 and 29 produced only 2 bands. However, the intensity of the bands are also more under fertile condition. In TGMS 6, POX 1, 2, 3, 5, 6 and 7 isoforms are seen in fertile condition. In TGMS 16 and TGMS 29 produced 6 and 5 numbers of isoforms *viz.*, 1, 2, 3, 4, 6 and 7 and 1, 5, 6 and 7 isoforms respectively. In sterile condition irrespective of the genotypes it produced 2 isoforms (POX 6 and 7).

4.3.3.2.1.3. Superoxide dismutase (SOD)

The polymorphism analysis of SOD enzyme revealed the presence of different isoforms in TGMS lines under fertile and sterile condition. There were 5 isoforms present in fertile TGMS lines depicted in the Table 49. One isoform SOD 5 was highly associated with fertility, with uniform high activity in TGMS line under fertile condition and in normal variety. The sterile genotypes produced only 3 isoforms *viz.*, SOD 1, 2 and 4

(TGMS 6) or SOD 1, 2 and 3 (TGMS 16) or SOD 1, 3, and 4 (TGMS 29) whereas fertile genotypes exhibited 5 isoforms *viz.*, SOD 1, 2, 3, 4 and 5 (Plate 7).

4.3.3.2.2. Esterase (EST)

The isozyme pattern of esterase enzyme revealed the presence of different isoforms in TGMS lines and a normal variety. Difference in number of bands between sterile and fertile TGMS lines were observed in the Plate 8 are tabulated in Table 49. Genotype TGMS 29 had given maximum number of isoforms for esterase enzyme (4) and TGMS 6 and TGMS 16 produced 3 isoforms under fertile condition. The sterile genotypes exhibited only one (TGMS 6) or 2 (TGMS 16 and TGMS 29) isoforms. The normal variety ADT 36 also showed 3 isoforms for esterase enzyme.

From the Table 49, it was inferred that 3rd isoform is associated with fertility of TGMS lines. The intensity of this band was also high in fertile condition than sterile condition.

4.3.3.2.3. Poly phenol oxidase (PPO)

The distribution of PPO isoforms in Plate 9 are translated in Table 49. Irrespective of genotypes, the fertile lines had 3 isoforms whereas sterile lines had 2 isoforms. Here also, the 3rd isoform plays a crucial role in fertility restoration i.e., 3rd loci is very particular in its presence in fertile lines alone.

4.4. Morphological and anatomical changes associated with male sterility in TGMS lines

Distinct differences were exhibited by the anthers and pollen grains of TGMS lines under fertile and sterile conditions. The fertile anthers were yellow, large and plumpy whereas the sterile anthers were small, shrivelled and white coloured (Plate 10). When stained with Iodine potassium iodide (IKI) the fertile anthers were stained fully and turned blue, but sterile anthers did not take up the stain and remained white in colour.

Table 49. Isozyme analysis of various enzymes in TGMS lines under fertile and sterile conditions (Number of isoforms)

Lines	Catalase (CAT)		Peroxidase (POX)		Super oxide dismutase (SOD)		Esterase (EST)		Polyphenol oxidase (PPO)	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
TGMS 6	1 2 3	1	1 2 3 5 6 7	6 7	1 2 3 4 5	1 2 4	1 2 3	1	1 2 3	1 2
TGMS 16	2 3	1	1 2 3 4 6 7	6 7	1 2 3 4 5	1 2 3	1 3 5	1 5	1 2 3	1 2
TGMS 29	1 2 3	1 2	1 5 6 7	6 7	1 2 3 4 5	1 3 4	1 2 3 4	1 2	1 2 3	1 2
ADT 36	1 2 3	-	4 5 6 7	-	1 2 3 4 5	-	1 2 3 5	-	1 2 3	-
Total alleles	3		6		5		4		3	

Microscopic examination of the pollen grains of TGMS lines was performed. The pollen grains from fertile anthers were round shaped and took the IKI stain completely and remained blue in colour whereas the sterile pollen grains were yellow in color, irregular in shape and did not take up the stain (Plate 11).

The microtomy section of sterile and fertile anthers were also performed to study the anatomical changes during fertility transformation under high temperature condition. The paraffin wax sections were examined carefully under microscope. It was observed that in sterile anthers a few aborted pollen grains were present with thin, persistent tapetum layer or the anthers were remained empty without any pollen grain. In fertile anthers, round pollen grains with disintegrated tapetum were observed. The fertile anthers also possessed firm endothecium cells and outer walls which were absent in sterile anthers (Plate 11).

4.5. Chemical manipulation of fertility and sterility

4.5.1. Induction of male sterility

4.5.1.1. Screening chemicals / growth regulators for the induction of male sterility in TGMS lines

Tables 50, 51 and 52 indicate the percentage of spikelet sterility induced by chemicals / growth regulators sprayed at different stages of panicle development in TGMS lines TGMS 6, TGMS 16 and TGMS 29 respectively.

Treatments were given at different stages of panicle development in combinations with different concentrations of chemicals / growth regulators. This was done to fix the correct stage and optimum concentration for the induction of complete spikelet sterility in TGMS lines.

Among the treatments SA 800 ppm, MH 0.1 per cent and Ethrel 400 ppm consistently induced more than 99 per cent spikelet sterility in all the TGMS lines. Among these three treatments dramatic effect of SA 800 ppm was observed in recording the highest percentage of spikelet sterility (100%) in all the three TGMS lines sprayed at PI alone, PI + 5DA PMC and PI + PMC + 5DA PMC stages followed by MH 0.1 per cent sprayed at PMC + 5DA PMC and PI + PMC + 5DA PMC and PMC + 5DA PMC. Apart from these three treatments, ethrel 200 ppm and SA 600 ppm recorded significantly higher percentage (upto 90%) of spikelet sterility at PMC + 5DA PMC stages and ethrel 400 ppm at PI + PMC + 5DA PMC and PI + 5DA PMC respectively. Rest of the treatments induced less than 70 per cent sterility.

Results suggested that foliar spray of salicylic acid (800 ppm) at PI stage was very effective in inducing male sterility of TGMS lines followed by MH (0.1%) at PMC + 5DA PMC stage.

4.5.1.2. Effect of salicylic acid treatment on pollen sterility and seed set of TGMS lines

Per cent pollen sterility and per cent seed setting of TGMS lines by salicylic acid treatment under pot culture and field conditions were presented in Tables 53 and 54. In pot culture condition, salicylic acid treatment at PI stage, exhibited more than 99 per cent pollen sterility in TGMS lines (Plate 13). But under field condition, pollen sterility recorded 93.9 per cent when the plants were treated at PI stage. When the treatment was given as two sprays during panicle initiation and 5 DA PMC stages, the pollen sterility increased upto 100 per cent in TGMS 29 and 98.8 per cent in TGMS 6 (Fig. 24).

Table 50. Effect of chemicals and growth regulators on spikelet sterility (%) of TGMS 6 at different stages of spray

Treatment	PI alone	PI + PMC	PI + PMC + 5 DA PMC	PMC alone	PMC + 5 DA PMC	5 DA PMC	PI + 5 DA PMC	Mean
T ₁ - control	12.6	9.2	15.0	12.7	15.8	9.1	12.2	12.64
T ₂ - SA 400 ppm	86.4	86.2	96.2	81.0	76.8	74.6	90.2	85.14
T ₃ - SA 600 ppm	98.6	96.2	98.8	76.8	86.4	87.2	94.3	91.77
T ₄ - SA 800 ppm	99.8	99.1	100.0	86.3	94.5	92.4	100.0	96.92
T ₅ - MH 0.05%	74.2	80.0	88.4	68.0	78.4	84.2	82.0	79.85
T ₆ - MH 0.1%	99.8	100.0	100.0	83.2	82.0	94.0	99.0	95.14
T ₇ - Ethrel 200 ppm	87.4	72.5	76.6	62.3	78.5	86.4	97.2	80.64
T ₈ - Ethrel 400 ppm	98.5	90.2	99.7	70.6	78.4	74.0	98.0	87.07
Mean	82.11	80.18	85.18	68.5	74.31	75.81	84.43	

CD (P=0.05) T S T x S
1.124 1.051 2.974

Table 51. Effect of chemicals and growth regulators on spikelet sterility (%) of TGMS 16 at different stages of spray

Treatment	PI alone	PI + PMC	PI + PMC + 5 DA PMC	PMC alone	PMC + 5 DA PMC	5 DA PMC	PI + 5 DA PMC	Mean
T ₁ - control	18.0	12.6	16.8	9.6	11.6	8.0	11.4	12.64
T ₂ - SA 400 ppm	82.4	86.0	94.5	78.2	80.5	80.3	89.8	84.71
T ₃ - SA 600 ppm	88.7	90.5	93.4	80.1	88.2	87.8	92.5	88.42
T ₄ - SA 800 ppm	100.0	98.2	100.0	88.8	90.6	88.0	98.6	95.58
T ₅ - MH 0.05%	81.3	84.0	87.5	56.2	67.7	72.5	80.8	75.85
T ₆ - MH 0.1%	98.7	99.3	100.0	86.2	78.4	89.0	98.8	93.5
T ₇ - Ethrel 200 ppm	84.4	88.0	90.1	70.5	73.2	72.5	94.0	82.0
T ₈ - Ethrel 400 ppm	97.6	92.1	100.0	82.4	77.3	79.0	99.3	90.28
Mean	81.81	81.75	85.81	69.31	71.25	72.06	83.31	

CD (P=0.05) T S T x S
0.0844 0.789 2.232

Table 52. Effect of chemicals and growth regulators on spikelet sterility (%) of TGMS 29 at different stages of spray

Treatment	PI alone	PI + PMC	PI + PMC + 5 DA PMC	PMC alone	PMC + 5 DA PMC	5 DA PMC	PI + 5 DA PMC	Mean
T ₁ - control	4.0	7.5	8.0	6.2	4.1	2.7	2.6	5.01
T ₂ - SA 400 ppm	88.2	82.98	94.44	77.6	81.52	80.0	88.0	85.21
T ₃ - SA 600 ppm	96.5	95.05	100.0	80.0	88.5	72.0	96.0	92.0
T ₄ - SA 800 ppm	100.0	99.82	100.0	82.0	96.0	92.0	100.0	97.0
T ₅ - MH 0.05%	79.0	82.0	85.0	78.0	80.0	88.4	80.0	82.14
T ₆ - MH 0.1%	100.0	100.0	100.0	84.0	88.0	96.0	98.0	96.42
T ₇ - Ethrel 200 ppm	82.0	68.0	78.0	56.0	81.0	84.0	95.0	78.14
T ₈ - Ethrel 400 ppm	96.0	83.0	100.0	58.0	85.0	98.0	98.5	89.0
Mean	81.57	78.6	84.4	85.71	76.14	77.09	83.82	

CD (P=0.05) T S T x S
1.044 0.977 2.763

Table 53. Effect of salicylic acid treatment on pollen and spikelet sterility (%), Selfed and crossed seed set (%) of TGMS lines (Pot culture experiment)

TGMS lines	Pollen sterility (%)		Spikelet sterility (%)		Crossed seed set (%)		Selfed seed set (%)	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
TGMS 6	18.6	99.0	46.6	100.0	-	37.54	53.4	0
TGMS 16	16.0	98.8	54.7	100.0	-	34.40	45.3	0
TGMS 29	10.4	100.0	33.7	100.0	-	48.26	66.3	0
ADT 36	4.0	-	16.7	-	-	-	87.3	-
Mean	15.0	99.26	45.0	100.0	-	40.06	55.0	0

CD (P=0.05)

L	13.972	3.093	3.947	2.966
S	11.412	3.052	3.222	2.422
L x S	19.764	5.358	5.582	4.195

Table 54. Effect of Salicylic acid treatment on pollen sterility (%) and crossed seed set (%) of TGMS lines (field experiment)

TGMS lines	Pollen sterility (%)		Crossed seed set (%)	
	Treatment at PI stage	Treatment at PI + 5 DA PMC	Rope pulling method	Clipping method
TGMS 6	89.3	98.8	17.20	26.42
TGMS 29	98.6	100.0	23.68	33.30
Mean	93.9	95.1	20.44	29.86

CD (P=0.05)

L	2.463	3.226
S	2.386	4.623
L x S	3.973	7.028

Fig.24 Effect of salicylic acid treatment on pollen and spikelet sterility and seed set of TGMS lines

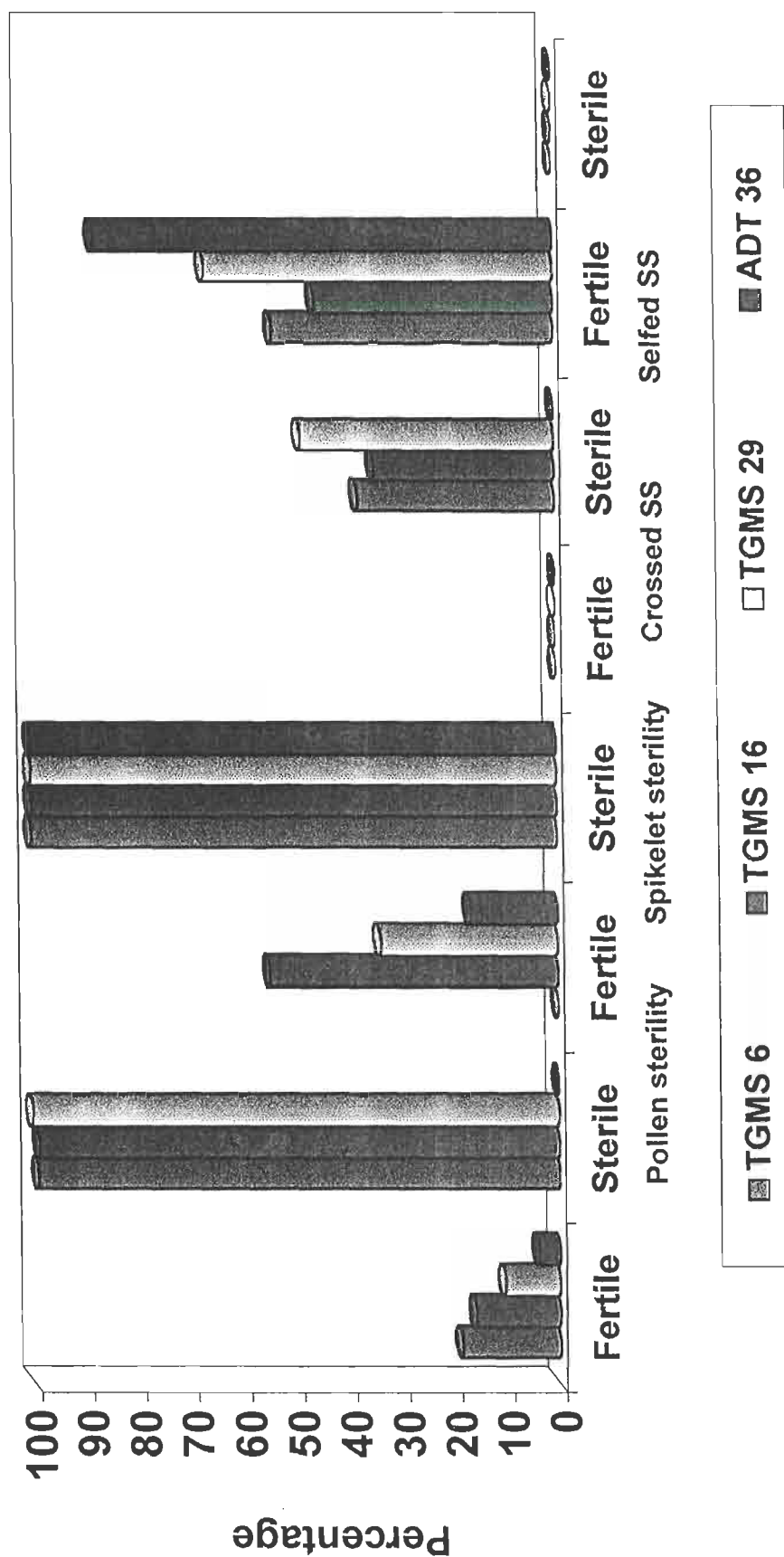
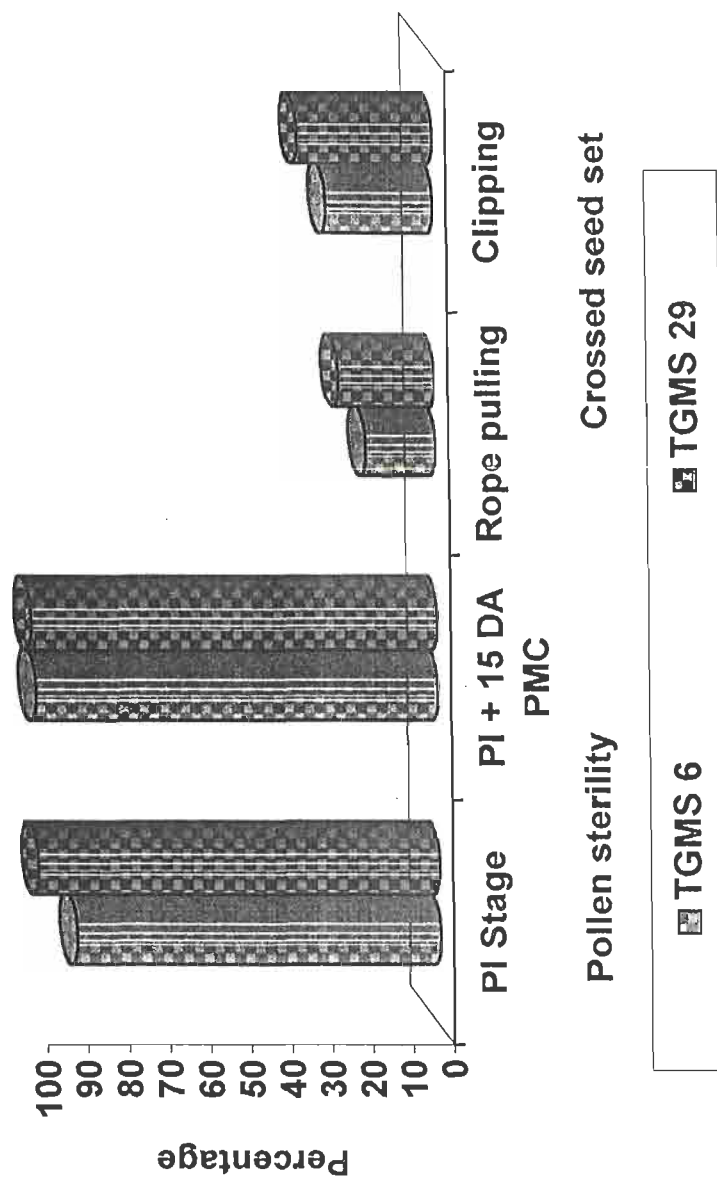


Fig.25 Effect of salicylic acid treatment on crossed seed set of TGMS lines



The per cent selfed seed setting of TGMS lines and a normal variety (ADT 36) was also calculated under fertile condition. The results showed that the highest seed setting percentage (87.3) was recorded by ADT 36 followed by TGMS 29 (66.3). Zero percent selfed seed setting was observed in TGMS lines under sterile condition (Fig. 25).

The crossed seed setting percentage also varied between pot culture and field conditions. In pot culture experiment, the mean value of crossed setting was 30 per cent. But in field experiment, the value recorded was 29.8 per cent by clipping method and 20.4 per cent by rope pulling method of pollination. The highest percentage of crossed seed setting was recorded by TGMS 29 (38.2) followed by TGMS 6 (27.5) by clipping and dusting method of crossing under pot culture condition. TGMS 29 also recorded the highest value (33.3 per cent) for crossed seed setting under field condition by clipping and dusting method. In rope pulling method, both the TGMS lines recorded only 20.4 per cent for crossed seed setting (Fig. 25). Highly significant differences were recorded between lines and also between treatments.

4.5.1.3. Effect of salicylic acid spray on biochemical parameters of TGMS lines

4.5.1.3.1. Soluble protein content

Table 55 indicates the effect of salicylic acid on soluble protein content of leaves at different stages of reproductive growth in TGMS lines. There were marked differences between treatments in soluble protein content but there was significant increase of soluble protein content over stages. The protein content reduced appreciably in treated plants with 8.3 per cent in 15 DA PI and 11.0 per cent in flowering stage (Fig. 26). There was a marginal variation between the TGMS lines. The mean protein content in control plants ranged from 7.03 mg g⁻¹ at panicle initiation stage to 12.89 mg g⁻¹ at flowering stage and

Table 55. Effect of salicylic acid spray on soluble protein content (mg g^{-1}) of TGMS lines

Stages Lines	PI			15 DA PI			Flowering		
	Control	Sprayed	% change	Control	Sprayed	% change	Control	Sprayed	% change
TGMS 6	6.92	6.81	-	12.02	11.16	8.0	12.58	12.20	11.0
TGMS 16	7.11	6.90	-	12.36	11.92	4.0	13.18	12.88	10.0
TGMS 29	7.08	6.77	-	12.62	11.03	13.0	12.91	11.37	12.0
Mean	7.03	6.95	-	12.33	11.37	8.33	12.89	11.48	11.0

CD ($P=0.05$) L S T L x S S x T L x T L x S x T
0.031 0.030 0.025 0.053 0.043 0.040 0.075

Table 56. Effect of salicylic acid spray on total carbohydrates content (mg g^{-1}) of TGMS lines

Stages Lines	PI			15 DA PI			Flowering		
	Control	Sprayed	% change	Control	Sprayed	% change	Control	Sprayed	% change
TGMS 6	74.21	72.67	-	93.16	80.47	14.0	96.92	81.52	16.0
TGMS 16	72.82	70.16	-	96.76	82.13	16.0	97.38	83.00	15.0
TGMS 29	72.07	69.48	-	95.32	80.03	17.0	97.87	83.17	16.0
Mean	73.03	72.70	-	95.08	80.87	15.33	97.39	82.56	15.66

CD ($P=0.05$) L S T L x S S x T L x T L x S x T
0.179 0.178 0.146 0.310 0.438

Fig.26 Effect of salicylic acid on soluble protein content of TGMS lines

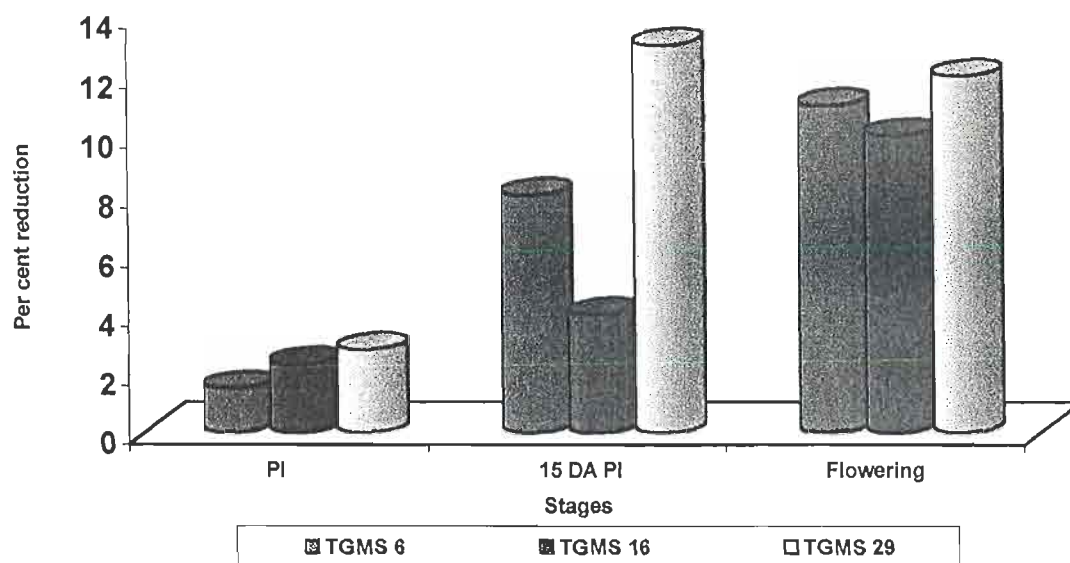
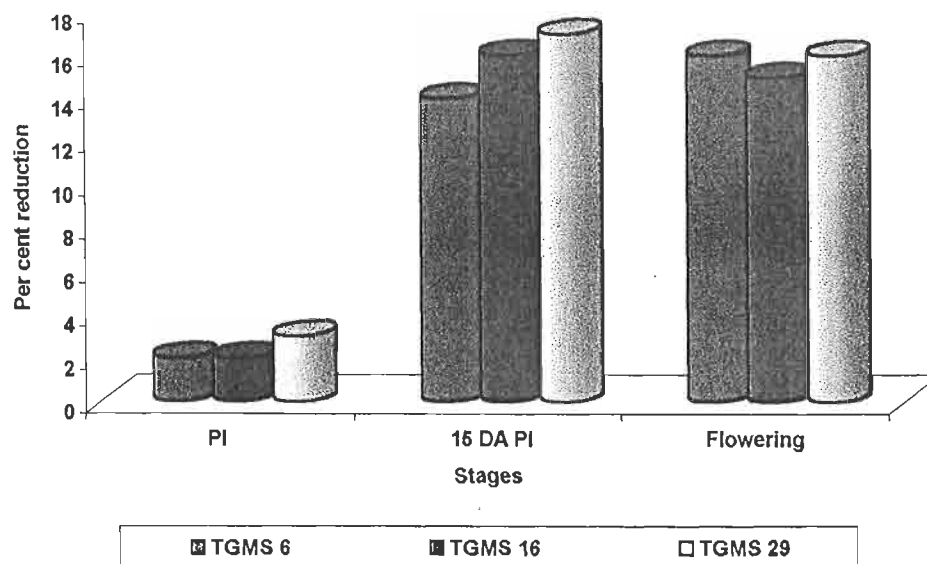


Fig. 27 Effect of salicylic acid on total carbohydrate content of TGMS lines



in salicylic acid treated plants it was ranged from 6.95 mg g^{-1} to 11.48 mg g^{-1} at PI and flowering stage respectively.

4.5.1.3.2. Carbohydrate fractions

4.5.1.3.2.1. Total carbohydrate content

Total carbohydrate content in leaves of salicylic acid treated TGMS lines compared with control plants is presented in Table 56.

Salicylic acid treatment significantly reduced the total carbohydrate content in leaves of treated plants compared to control. The mean values of total carbohydrate content for control plants were 73.03 mg g^{-1} , 95.08 mg g^{-1} and 97.39 mg g^{-1} at PI, 15 DA PI and flowering stage respectively and for treated plants it was from 72.7 mg g^{-1} at PI stage to 82.56 mg g^{-1} at flowering stage. The per cent reduction of total carbohydrates in treated plants was 15.33 at 15 DA PI and 15.66 at flowering stage (Fig. 27).

There was significant differences between treatments and between the stages. The interaction effects were also significant at all the stages except at PI stage.

4.5.1.3.2.2. Starch content

The effect of salicylic acid treatment on starch content of TGMS lines at PI, 15 DA PI and flowering stage is given in Table 57.

In both, control and salicylic acid treatments, the starch content significantly increased from stage 1 onwards but there was a significant difference between the treatments. The content of starch in salicylic acid treated plants was higher compared to control plants in all the stages of observation. The percentage of increase was 21.6 at 15 DA PI and 29.0 at flowering over the control but it was negligible at PI stage because

Table 57. Effect of salicylic acid spray on starch content (mg g^{-1}) of TGMS lines

Stages Lines	PI			15 DA PI			Flowering		
	Control	Sprayed	% change	Control	Sprayed	% change	Control	Sprayed	% change
TGMS 6	26.71	26.76	-	32.00	40.61	22.0	30.76	42.22	28.0
TGMS 16	26.60	27.13	-	31.88	42.11	25.0	31.60	44.30	29.0
TGMS 29	28.82	28.64	-	34.16	41.38	18.0	31.33	44.16	30.0
Mean	27.37	27.51	-	32.68	41.36	21.66	31.22	43.56	29.0

CD (P=0.05) L S T L x S S x T L x T L x S x T
0.037 0.036 0.029 0.062 0.051 0.050 0.088

Fig.28 Effect of salicylic acid on starch content of TGMS lines

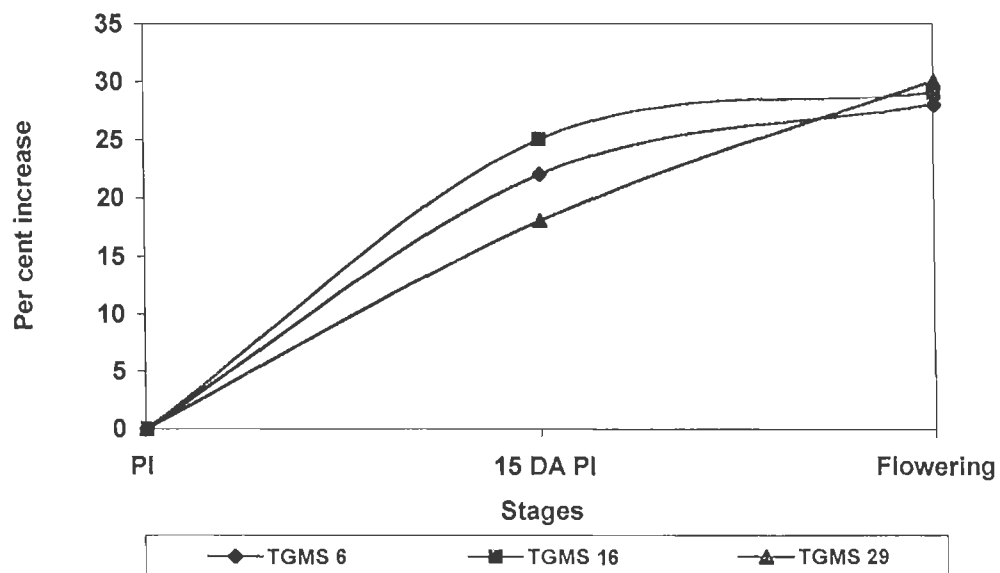
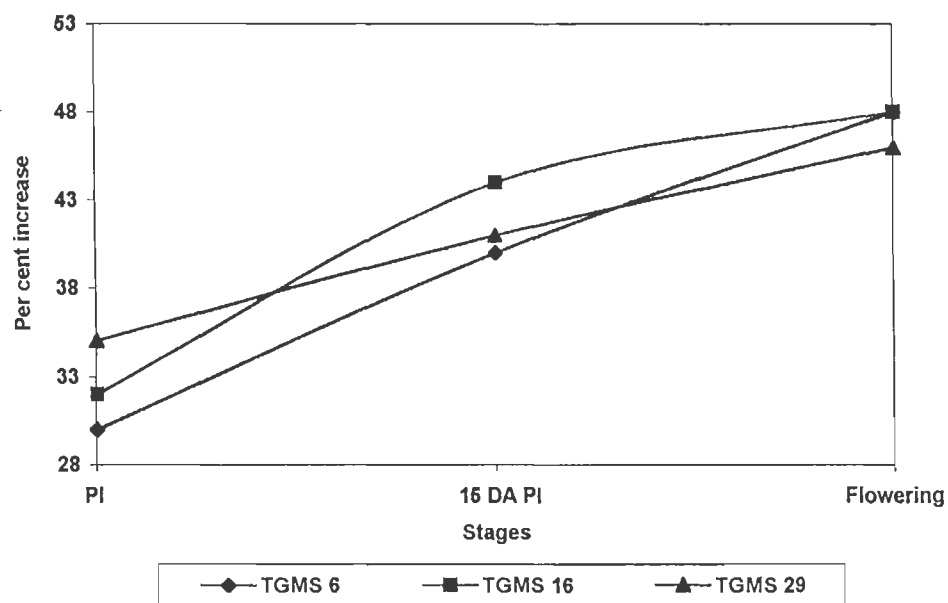


Fig.29 Effect of salicylic acid on total phenol content in TGMS lines



the samples were drawn immediately after spray (Fig. 28). The mean values of starch content in treated plants were 27.51 mg g^{-1} 41.36 mg g^{-1} and 43.56 mg g^{-1} at PI, 15 DA PI and flowering stage respectively.

The differences between treatments and between stages were significant. Except PI stage, all the stages showed significant interaction effects.

4.5.1.3.3. Nucleic acids

4.5.1.3.3.1. Deoxyribo nucleic acid (DNA) content

The deoxy ribonucleic acid content of three TGMS lines is given in Table 58.

1

The salicylic acid treated plants showed a significant decrease in DNA content in all the stages over its control. The maximum content of $651.0 \text{ } \mu\text{g g}^{-1}$ was recorded in control during 15 DA PI and lowest of $492.8 \text{ } \mu\text{g g}^{-1}$ of DNA during PI stage. In treated plants the DNA content ranged from 489.6 to $542.9 \text{ } \mu\text{g g}^{-1}$. Flowering stage recorded the peak DNA content of $542.9 \text{ } \mu\text{g g}^{-1}$ whereas PI stage recorded a lowest of $489.6 \text{ } \mu\text{g g}^{-1}$ of DNA content among its various phenological stages.

The per cent decrease was more during 15 DA PI (18.3%) followed by flowering stage (16.3%) and during PI stage it was negligible over its absolute control. The difference between the treatments, stages, and their interactions were significant.

4.5.1.3.3.2. Ribo nucleic acid (RNA) content

The data on RNA content is depicted in Table 59.

The salicylic acid treated plants showed a significant decrease in RNA content in all the stages. The control plants showed an increasing trend throughout their stages. There was a significant difference for the treatment. The maximum RNA content was recorded during flowering stage ($598.4 \text{ } \mu\text{g g}^{-1}$) in control plants and lowest of $546.7 \text{ } \mu\text{g g}^{-1}$

Table 58. Effect of salicylic acid spray on DNA content ($\mu\text{g g}^{-1}$) of TGMS lines

Stages Lines	PI			15 DA PI			Flowering	
	Control	Sprayed	% change	Control	Sprayed	% change	Control	Sprayed
TGMS 6	482.1	480.6	-	632.6	523.8	18.0	630.3	530.8
TGMS 16	487.7	486.8	-	657.0	527.6	20.0	638.4	541.3
TGMS 29	508.7	501.6	-	663.4	552.0	17.0	667.1	556.6
Mean	492.8	489.6	-	651.0	537.4	18.3	645.2	542.9

CD (P=0.05) L S T L x S S x T L x T L x S x T
0.125 0.119 0.094 0.199 0.166 0.161 0.282

Table 59. Effect of salicylic acid spray on RNA content ($\mu\text{g g}^{-1}$) of TGMS lines

Stages Lines	PI			15 DA PI			Flowering	
	Control	Sprayed	% change	Control	Sprayed	% change	Control	Sprayed
TGMS 6	556.7	548.7	-	582.4	506.1	18.0	616.5	492.3
TGMS 16	540.3	539.6	-	577.5	491.8	20.0	587.6	480.1
TGMS 29	543.1	540.1	-	578.0	487.3	17.0	591.3	483.8
Mean	546.7	542.8	-	651.0	537.4	18.3	645.2	542.9

CD (P=0.05) L S T L x S S x T L x T L x S x T
0.033 0.031 0.025 0.054 0.044 0.040 0.076

at PI stage. In treated plants, RNA content ranged from 485.4 to 542.8 $\mu\text{g g}^{-1}$. Highest and lowest content was recorded at PI and flowering stages respectively. The per cent decrease in treated plants was 15.0 at 15 DA PI and 22.0 at flowering stage over its control. There was a significant difference between treatments, stages and their interactions.

4.5.1.3.4. Proline content

Proline content of three TGMS lines treated with salicylic acid is given in Table 60. There was a gradual increase in proline content upto 15 DA PI, after that it showed a declining trend. Due to salicylic acid application, proline content was increased from 0.85 mg g^{-1} to 4.77 mg g^{-1} at PI and 15 DA PI stages respectively in TGMS lines.

Irrespective of the lines, the per cent increase in proline content over control ranged from 66.6 per cent to 70.6 per cent at flowering and 15 DA PI stages respectively (Fig. 31). The mean values of proline content was 0.84, 1.4 and 1.42 mg g^{-1} for control plants at PI, 15 DA PI and flowering stages respectively and 0.85, 4.77 and 4.17 mg g^{-1} for treated plants at PI, 15 DA PI and flowering stages respectively. There was significant difference observed between treatments and also between stages.

4.5.1.3.5. Total phenol content

Table 61 shows the total phenol content of salicylic acid treated and untreated plants of TGMS lines at PI, 15 DA PI and flowering stages.

Total phenol content increased over stages in both the treatments in all the TGMS lines but the percentage of increase was significantly more in salicylic acid treated plants compared to control ones (Fig. 29). The per cent increase was 41.6 at 15 DA PI and 47.3 at flowering stage in treated plants but at PI stage it was negligible because the samples were taken immediately after the treatment. The increase in total phenol content was from

Table 60. Effect of salicylic acid spray on proline content (mg g^{-1}) of TGMS lines

Stages Lines	PI			15 DA PI			Flowering		
	Control	Sprayed	% change	Control	Sprayed	% change	Control	Sprayed	% change
TGMS 6	0.78	0.76	-	1.35	4.82	72.0	1.37	4.31	69.0
TGMS 16	0.91	0.90	-	1.47	4.88	70.0	1.39	4.07	66.0
TGMS 29	0.83	0.88	-	1.40	4.63	70.0	1.51	4.08	63.0
Mean	0.84	0.85	-	1.40	4.77	70.6	1.42	4.17	66.0

CD (P=0.05) L S T L x S S x T L x T L x S x T
0.031 0.030 0.025 0.053 0.043 0.040 0.075

Table 61. Effect of salicylic acid spray on total phenol content (mg g^{-1}) of TGMS lines

Stages Lines	PI			15 DA PI			Flowering		
	Control	Sprayed	% change	Control	Sprayed	% change	Control	Sprayed	% change
TGMS 6	1.80	2.68	-	2.28	3.80	40.0	2.93	5.62	48.0
TGMS 16	2.28	3.47	-	2.45	4.30	44.0	3.52	6.06	48.0
TGMS 29	1.71	2.82	-	2.16	3.62	41.0	3.10	5.66	46.0
Mean	1.93	1.99	-			41.6	3.18	5.98	47.3

CD (P=0.05) L S T L x S S x T L x T L x S x T
0.036 0.035 0.029 0.062 0.054 0.051 0.089

1.99 mg g⁻¹ to 5.98 mg g⁻¹ at PI stage and flowering stage respectively in treated plants but in control plants it was from 1.93 mg g⁻¹ to 3.18 mg g⁻¹ at PI and flowering stage respectively. The differences between treatments and between stages were significant except in PI stage.

4.5.1.3.6. IAA oxidase activity

The data on IAA oxidase activity in TGMS lines treated with salicylic acid is presented in Table 62.

IAA oxidase activity in control plants showed an increasing trend upto 15 DA PI and then it started declining. But in treated plants, IAA oxidase activity showed a gradual increase over the stages. There existed a significant difference for treatment, stages and their interactions.

The per cent increase in activity was 20.0 during 15 DA PI and 37.0 during flowering stage in treated plants over their control plants (Fig. 30). The control plants recorded the mean values of 16.64, 16.97 and 17.21 µg of unoxidised auxin at PI, 15 DAPI and flowering stages, respectively. In treated plants it was 16.47, 13.66 and 10.66 µg unoxidised auxin at PI, 15 DAPI and flowering stages respectively.

4.5.1.3.7. Ascorbic acid oxidase activity

Data are presented in Table 63. In control plants the ascorbic acid oxidase enzyme activity was ranged from 2.33 to 2.83 enzyme units g⁻¹. In treated plants it ranged between 2.42 and 6.03 enzyme units g⁻¹. In control plants the activity was maximum at 15 DA PI, later it showed a declining pattern, but in treated plants the activity showed an increasing trend upto flowering stage.

Table 62. Effect of salicylic acid spray IAA oxidase activity of TGMS lines (μ moles of unoxidised auxin $\text{h}^{-1} \text{g}^{-1}$)

Stages Lines	PI			15 DA PI			Flowering	
	Control	Sprayed	% change	Control	Sprayed	% change	Control	Sprayed
TGMS 6	17.12	16.96	-	17.43	14.11	20.0	17.38	11.46
TGMS 16	17.03	16.97	-	17.22	13.70	21.0	17.25	11.30
TGMS 29	15.78	15.50	-	16.27	13.18	19.0	17.02	10.12
Mean	16.64	16.47	-	16.97	13.66	20.0	17.21	10.96
CD (P=0.05)								
	L	S	T	L x S	S x T	L x T	L x S x T	
	0.031	0.030	0.024	0.052	0.043	0.040	0.074	

Table 63. Effect of salicylic acid spray on ascorbic acid oxidase activity (Enzyme units g^{-1}) of TGMS lines

Stages Lines	PI			15 DA PI			Flowering	
	Control	Sprayed	% change	Control	Sprayed	% change	Control	Sprayed
TGMS 6	2.16	2.20	-	2.78	5.67	51.0	2.52	6.16
TGMS 16	2.42	2.44	-	2.88	5.32	46.0	2.38	5.92
TGMS 29	2.41	2.58	-	2.85	5.40	48.0	2.41	6.02
Mean	2.33	2.40	-	2.83	5.46	48.3	2.43	6.03
CD (P=0.05)								
	L	S	T	L x S	S x T	L x T	L x S x T	
	0.276	0.270	0.228	0.479	0.391	0.382	0.677	

Fig.30 Effect of salicylic acid on IAA oxidase activity of TGMS lines

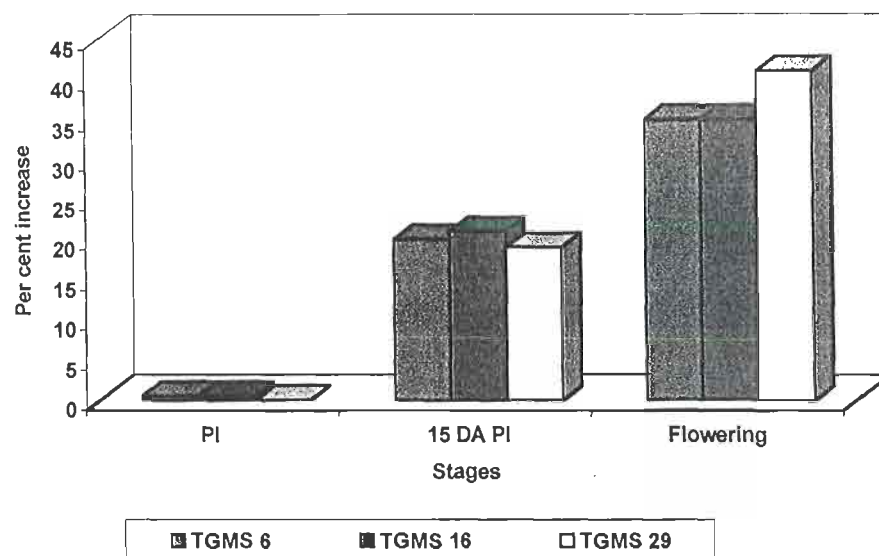
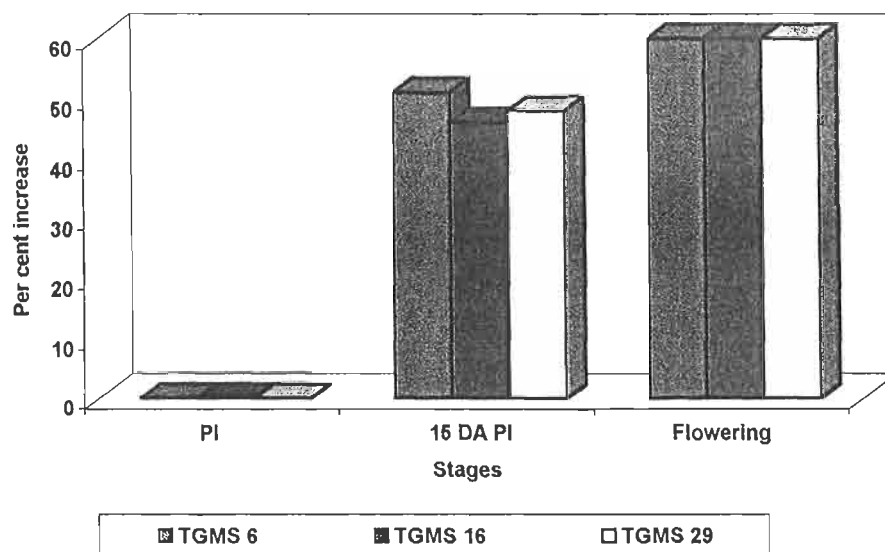


Fig.31 Effect of salicylic acid on AAO activity of TGMS lines



The difference between the activity of ascorbic acid oxidase in treated and control plants was significant during 15 DA PI and flowering stages only. The per cent increase due to application of salicylic acid, ranged from 48.3 to 60.0 per cent at 15 DA PI and flowering stages respectively (Fig. 31).

4.5.1.3.8. Catalase (CAT) activity

Table 64 shows data on catalase activity of TGMS lines treated with salicylic acid and its control.

There was a significant increase in catalase activity upto 15 DAPI after that the activity reduced in both the treatments. The control plants exhibited significantly higher catalase activity compared to salicylic acid treated plants. The per cent reduction due to treatment was negligible at PI stage, 43.0 at 15 DAPI and 41.6 at flowering stage over its control. The maximum activity of catalase recorded ($3.6 \text{ enzyme units} \times 10^4 \text{ g}^{-1}$) during 15 DA PI stage in control plants and the least activity was at flowering stage ($2.05 \text{ enzyme units} \times 10^4 \text{ g}^{-1}$) in treated plants.

4.5.1.3.9. Peroxidase activity

The data on peroxidase activity of TGMS lines showed a decreasing trend due to salicylic acid treatment (Table 65). But there existed an increase in activity upto 15 DA PI in control plants after that it remained the same. In treated plants, the reduction of catalase activity ranged from 20.3 per cent during flowering stage to 21.6 per cent at 15 DA PI stage over its control (Fig. 32). In control TGMS lines, the activity ranged from $157.6 \text{ enzyme units l}^{-1} \text{ h}^{-1}$ at PI stage to $179.2 \text{ enzyme units l}^{-1} \text{ h}^{-1}$ at flowering stage. In salicylic acid treated TGMS lines the activity reduced from $156.6 \text{ enzyme units l}^{-1} \text{ h}^{-1}$ at PI stage to $141.0 \text{ enzyme units l}^{-1} \text{ h}^{-1}$ at 15 DA PI stage.

Table 64. Effect of salicylic acid spray on catalase activity (Enzyme units $\times 10^4 \text{ g}^{-1}$) of TGMS lines

Stages Lines	PI			15 DA PI			Flowering		
	Control	Sprayed	% change	Control	Sprayed	% change	Control	Sprayed	% change
TGMS 6	2.88	2.43	-	3.51	2.02	43.0	3.52	2.02	43.0
TGMS 16	2.70	2.31	-	3.62	2.11	42.0	3.43	2.04	41.0
TGMS 29	2.94	2.44	-	3.68	2.07	44.0	3.54	2.10	41.0
Mean	2.81	2.78	-	3.60	2.06	43.0	3.49	2.05	41.6

CD (P=0.05) L 0.036 S 0.032 T 0.025 L x S 0.053 S x T 0.048 L x T 0.046 L x S x T 0.075

Table 65. Effect of salicylic acid spray on peroxidase activity (Enzyme units $l^{-1} h^{-1}$) of TGMS lines

Stages Lines	PI			15 DA PI			Flowering		
	Control	Sprayed	% change	Control	Sprayed	% change	Control	Sprayed	% change
TGMS 6	154.3	153.7	-	176.4	142.4	20.0	173.1	142.6	18.0
TGMS 16	156.2	156.0	-	176.8	140.3	21.0	176.1	141.5	20.0
TGMS 29	162.3	157.3	-	184.4	140.4	24.0	183.4	142.7	23.0
Mean	157.6	156.6	-	179.2	141.0	21.6	177.5	142.2	20.3

CD (P=0.05) L S T L x S S x T L x T L x S x T
 1.368 1.355 1.115 2.365 1.937 1.928 3.345

Table 66. Effect of salicylic acid spray on SOD activity (Enzyme units mg^{-1} protein h^{-1}) of TGMS lines

Stages Lines	PI			15 DA PI			Flowering		
	Control	Sprayed	% change	Control	Sprayed	% change	Control	Sprayed	% change
TGMS 6	7.61	7.56	-	8.22	6.82	18.0	8.20	6.32	23.0
TGMS 16	7.32	7.31	-	8.17	6.34	23.0	8.11	6.17	25.0
TGMS 29	8.02	7.96	-	8.40	6.77	20.0	8.26	6.20	30.0
Mean	7.65	7.61	-	8.26	6.64	20.3	8.19	6.23	26.0

CD (P=0.05) L S T L x S S x T L x T L x S x T
 0.329 0.319 0.267 0.567 0.463 0.463 0.802

Fig. 32 Effect of salicylic acid on peroxidase activity of TGMS lines

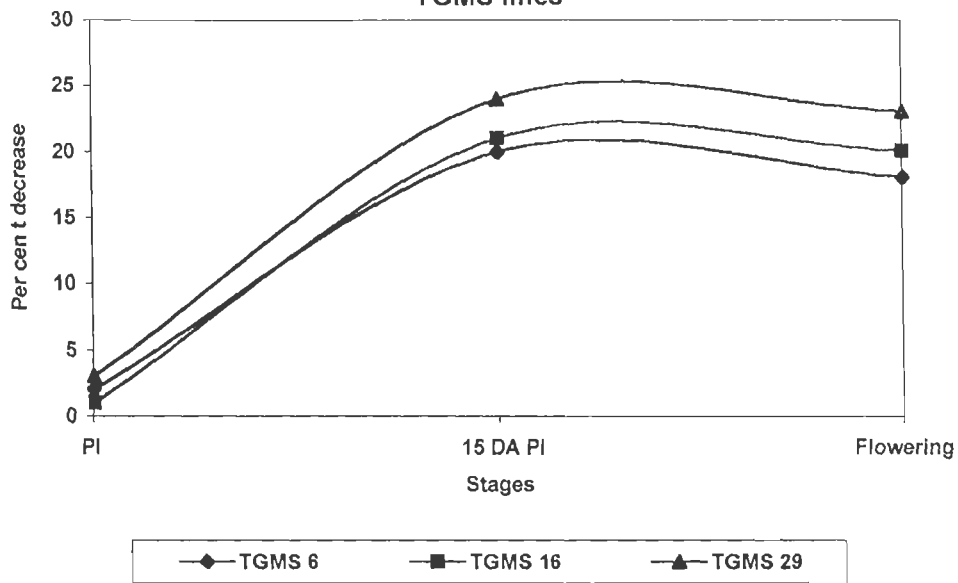
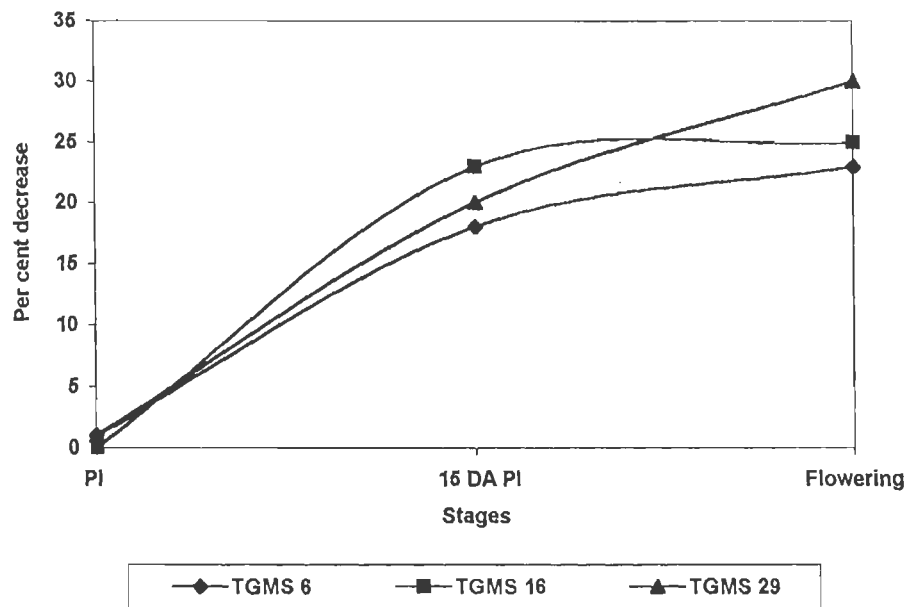


Fig.33 Effect of salicylic acid on SOD activity of TGMS lines



4.5.1.3.10. Superoxide dismutase (SOD) activity

Table 66 shows the superoxide dismutase (SOD) activity of salicylic acid treated TGMS lines at different stages of panicle development.

In both the treatments, the activity of SOD increased upto 15 DA PI and then it started declining. Irrespective of the genotypes, the maximum activity recorded at 15 DA PI (8.26 enzyme units mg^{-1} protein h^{-1}) followed by flowering stage (8.19 enzyme units mg^{-1} protein min^{-1}) in control plants. In treated plants, the maximum activity recorded during PI stage (7.61 enzyme units mg^{-1} protein min^{-1}). There was a significant reduction of SOD activity in treated plants over its control plants. The per cent reduction was 20.3 and 26.0 at 15 DA PI and flowering stages respectively (Fig. 33). There existed a significant difference between treatments, stages and their interactions.

4.5.2. Induction of fertility

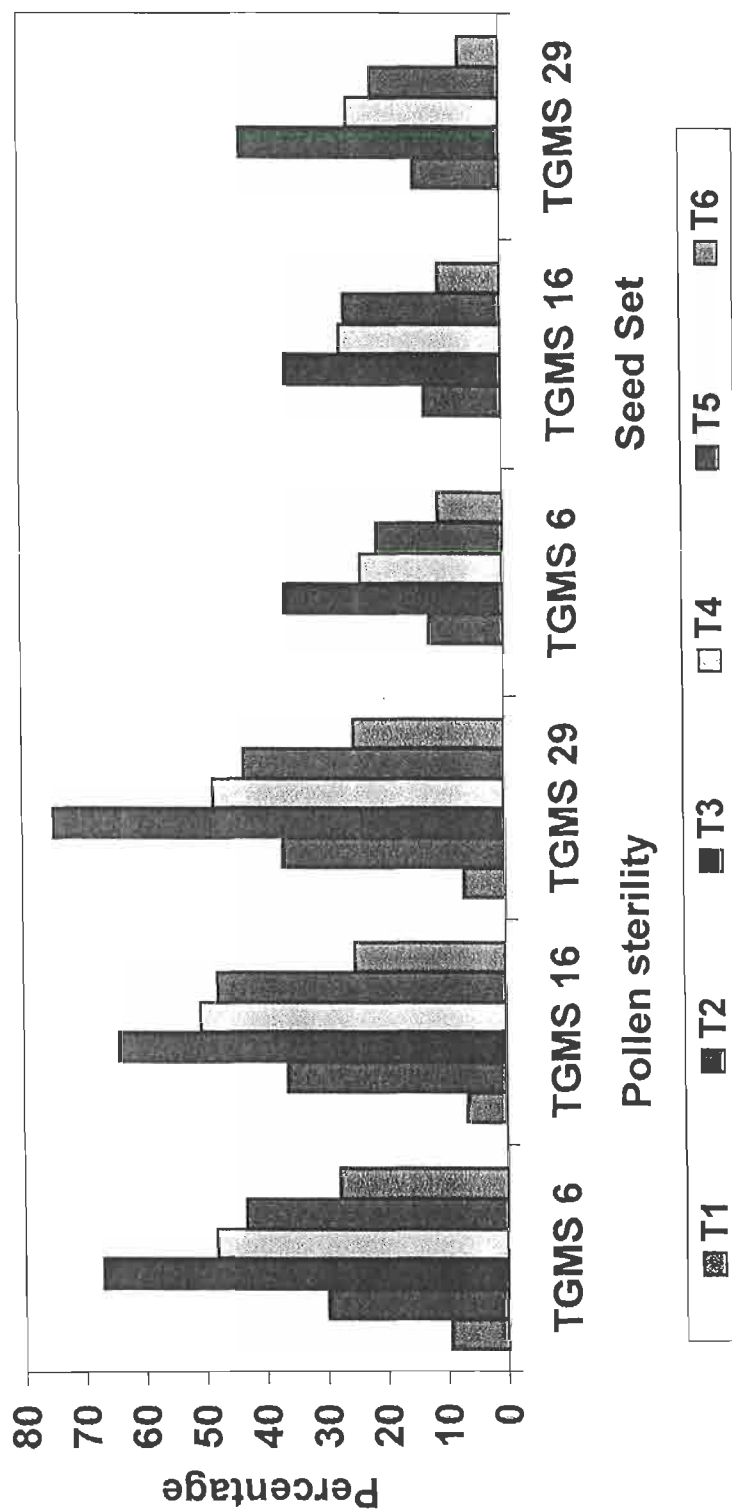
The efficiency of plant growth hormones and regulators on the induction of male fertility in TGMS lines is presented in Table 67. Their efficiency was tested by assessing per cent pollen fertility and per cent spikelet fertility (selfed seed set) in TGMS lines. Among various treatments, spectacular increase was observed in recording higher percentage of pollen fertility (65.9%) in IAA (50 ppm) sprayed plants and selfed seed set percentage (38.5%) followed by brassinosteroid (BR-1 ppm) treatment (49.0% and 25.2 %). NAA (50 ppm) spray also induced significantly higher pollen fertility (44.4%) and selfed seed set percentage (22.6%) but it stood next to BR (1 ppm). Rest of the treatment showed significantly lower percentage of pollen fertility and selfed seed set compared to IAA and BR. The control plants showed complete pollen sterility. Results suggested that IAA (50 ppm) and BR (1 ppm) were more effective in increasing seed set of TGMS lines by inducing pollen fertility under high temperature conditions (Fig. 34 and Plate 16).

Table 67. Induction of fertility in TGMS lines by chemicals and plant growth regulators

Treatments (ppm)	TGMS 6		TGMS 16		TGMS 29		Mean	
	Pollen fertility (%)	Seed set (%)	Pollen fertility (%)	Seed set (%)	Pollen fertility (%)	Seed set (%)	Pollen fertility (%)	Seed set (%)
T ₁ – Control	9.6	0	6.5	0	6.9	0	7.6	0
T ₂ – Ascorbic acid (100)	29.8	12.3	36.3	12.8	36.9	14.4	34.3	13.16
T ₃ – IAA (50)	66.9	36.4	64.0	36.0	74.9	43.1	65.9	38.50
T ₄ – BR (1)	48.1	23.6	50.7	26.8	48.3	25.3	49.03	25.23
T ₅ – NAA (50)	43.2	21.0	47.9	26.0	43.2	21.4	44.4	22.80
T ₆ – GA (50)	27.8	10.8	25.9	10.4	25.0	5.8	22.9	9.08
Mean								

CD (P=0.05) Pollen fertility L T L x T
Seed set 2.67 3.82 5.84
2.18 3.38 5.23

Fig.34 Induction of fertility in TGMS lines by growth regulators



Discussion

CHAPTER V

DISCUSSION

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Environmental factors profoundly influence the action of many sterility causing genes, so that the male sterility expression of the genotypes is related to the environmental conditions in which they are grown. Except in a few cases, the precise determination of the effect of specific environmental factors on male sterility gene expression has not been investigated. Whereas many of these genes are susceptible to temperature, photoperiod, light intensity or to some other undetermined environmental factors, a few seem to exhibit stability to some of these environmental components. In TGMS lines, recessive nuclear gene controlled the male sterility, which is heritable, but the expression of the gene is influenced by temperature at specific stage of panicle development. When the temperature stimulus is perceived, a signal will be generated and transmitted and a biochemical change will be instigated to cause the response. The speed, sensitivity and specificity of this stimulus response are controlled by a network of positively and negatively acting elements. These elements are responsible for ensuring a tissue specific response that is quantitatively appropriate, correctly timed and highly co-ordinated. Thus a wide range of studies on physiological, biochemical, molecular and anatomical aspects of male sterility have been instrumental in understanding the mechanism of TGMS in rice.

With this objectives, the present investigation was carried out to study the critical temperature and the critical stage at which thermosensitive genic male sterile lines can alter their male fertility and to understand the various physiological, biochemical and molecular mechanisms causing male sterility. Understanding the accumulation or depletion of different physiological or biochemical compounds during fertility / sterility, will be useful to manipulate the sterility / fertility by applying those chemicals or growth

regulators exogenously. This would aid in obtaining 100 percent sterile TGMS lines even under unfavourable weather conditions. Development of stable pollen sterile TGMS lines in turn would help the breeders to exploit maximum heterotic vigour in two-line hybrid rice breeding. The results of various experiments conducted to apply the above objectives are discussed in this chapter with sufficient proof from earlier studies.

5.1 Critical Sterility Temperature (CST)

Thermosensitive genic male sterility (TGMS) is a type of genetic male sterility expression, which is regulated by certain temperature conditions. TGMS lines have complete pollen sterility at 30~34°C / 22~23°C and partial to normal pollen sterility at 24~27°C / 18~19°C (Sun *et al.*, 1989; Virmani and Voc, 1991). Successful use of TGMS lines in hybrid rice breeding depends on their stability and adaptability across environments, since the nuclear sterile gene reacts differently to temperature under different genetic backgrounds (Lu *et al.*, 1994; Virmani, 1994). Yuan (1992) suggested that the CST- the minimum temperature for complete pollen sterility varies from 23°C to 29°C depending on the TGMS lines. In the present investigation, CST of selected TGMS lines was identified by subjecting them to different daily mean temperatures during panicle development stages using phytotron. The pollen sterility rate was in accordance with the temperature. The CST was recorded as 28.0°C for TGMS 6, 27.0°C for TGMS 16 and 27.5°C for TGMS 29, which were in line with the results of Yuan (1992) for TGMS lines.

In the present study, the maximum / minimum temperature for the complete pollen sterility in TGMS 6 was 34/22°C, TGMS 16 was 33/21°C and TGMS 29 was 34/21°C which confirmed the findings of Borkakati and Virmani (1996). The first TGMS line, Annong 1S had a CST of 27.0°C (Lu *et al.*, 1994). Virmani (1992) has reported the CST of Norin PL 12 - which was the source of male sterility for TGMS 16 and TGMS

18, as a mean daily temperature of 27.5°C (31° / 24°C). This corroborated the findings of the present experiment. Gong *et al.* (2000) has reported a similar maximum / minimum temperature range of 34/22°C for a TGMS line Ping Xiang under phytotron condition, for complete pollen sterility. Shu and Chen (1999) reported 31/28°C as daily mean temperature for Heng Wong S2 and 1356S, which showed complete pollen sterility at Huarihua region in China. Roystephen and Thangaraj (2000) identified CST for TGMS 16 (26.5°C) and TGMS 18 (27.5°C) under phytotron condition. These results were in conformity with the present study.

5.2. Critical thermosensitive stage

Knowledge on the critical thermosensitive stage for fertility alteration of TGMS lines is useful to determine most suitable time of their planting for multiplication or hybrid seed production. Conditions inducing fertility alteration in EGMS lines vary among different lines due to different source of male sterile genes and the effect of genetic backgrounds (Zhang *et al.*, 1992). Yuan *et al.* (1988) identified the stage from secondary rachis branching and spikelet primordial differentiation to pollen mother cell formation in the process of panicle development as the sensitive stage for fertility alteration in Nongken 58S. Zhang and Lu (1992a) reported that pollen sterility of some TGMS lines was positively correlated with the daily air temperatures at around 10 to 16 days before heading.

Zhang *et al.* (1993) also reported the stages between the formation of secondary rachis branch and the formation of PMC as the critical stage of thermosensitivity in TGMS lines. But Wang *et al.* (1991a, b) have contradicted this by reporting that the stamen and pistil primordia differentiation was more critical than panicle branch primordia and PMC meiosis stage. The critical thermosensitive stage in the present investigation was found to be 10-25 days before heading (DBH) as it was the minimum

period of exposure to CST to get complete pollen sterility and the sterility per cent reduced when the plants were exposed to CST at 10-20 DBH or 5-20 DBH. Maruyama *et al.* (1990) found that the critical stage for Norin PL 12, the source of male sterility for TGMS lines 16 and 18, as 22 to 26 DBH and that even high temperature for an hour would adversely affect the seed setting. Zeng (1991) reported that the daily mean temperature during 13 to 25 DBH and during 10 to 18 DBH were crucial for complete pollen sterility. This was in accordance with the findings of present experiment.

In the present investigation, 10-25 DBH was identified as critical thermosensitive stage for fertility alteration, which corresponded to differentiation of secondary branch primordial, differentiation of pistil and stamen, formation of PMC, and early meiosis stages (Stage III to IV). This corroborated the findings of Cheng *et al.* (1996); Laskhmi Praba and Thangaraj (2000) and Gong *et al.* (2000). Yao *et al.* (1995) reported that 5-20 DBH was critical stage in TGMS lines. But in the present study, the sterility was only 90 percent when exposed to 5-20 DBH. This might be due to the variation in thermosensitivity between TGMS lines as reported by Ali *et al.* (1995) that sensitive stage varied according to genotypes. Yang *et al.* (1995) reported that there were two important stages of thermosensitivity in TGMS lines, one at about 20 DBH around PMC stage and another at meiosis stage. This finding holds well in the present study also.

From the present findings, it may be concluded that the critical thermosensitive stage of the selected TGMS lines is from 10 to 25 DBH as it is the minimum period of exposure to CST to get complete pollen sterility.

5.3. Physiological, biochemical and molecular changes associated with male sterility in TGMS lines

The overall pattern of plant growth and development are directed by two classes of regulatory signals *viz.*, ontogenic signals programmed by the genome and environmental signals that provide external cues for continued action of the ontogenic signals. Response to developmental and environmental cues occur by stimulus - response coupling. When a stimulus is perceived, a signal will be generated and transmitted and a biochemical change will be instigated to cause the response. The external factors are responsible for the expression or suppression of TGMS gene(s), which will alter the internal mechanism of the plant. Keeping this in mind, various physiological, biochemical and molecular aspects were analyzed during the thermosensitive stages of fertility alteration under fertile and sterile conditions, to study the exact mechanism causing male sterility in TGMS lines. The change in physiological and biochemical parameters during fertility alteration is discussed here with probable contribution of each factor towards causing male sterility in TGMS lines of rice.

5.3.1. Physiological changes

5.3.1.1. Gas exchange measurements

Photosynthesis is the single most important physico-biochemical process of the world, on which the existence of life on earth depends, which is affected by temperature variation. The rate of photosynthesis of TGMS lines reduced significantly in high temperature induced sterile condition. The results of present study on transpiration rate and stomatal diffusive resistance clearly confirmed the effect of high temperature on photosynthetic rate was not due to stomatal limitation but due to mesophyll limitation. At lower or higher temperatures carbon exchange rate was reduced, thereby the net photosynthetic rate was affected (Kabede *et al.*, 1989). Pastense and Horton (1996) found the reduction of photosynthesis due to higher temperatures in beans. They reported that the limitation in the supply of reducing power was the main cause for the reduction in

photosynthesis than reduced stomatal conductance. A change in the content or activity of the mesophyll enzyme Rubisco will also alter the photosynthetic rate in plants. Roystephen (1998) reported the reduction in Rubisco content (42%) in sterile TGMS lines due to high temperature. Thus, the reduction in Rubisco content would have contributed directly for the reduction in photosynthetic rate of TGMS lines under sterile condition. Maruyama *et al.* (1990) also reported that the synthesis of Rubisco and several thylakoid proteins responsible for photosynthetic electron transport and photo phosphorylation was greatly inhibited at high temperatures.

5.3.1.2. Hill reaction

The photochemical efficiency leading to the production of assimilatory power measured as Hill reaction reduced upto 24 percent in sterile TGMS lines compared to fertile plants. Similar results were reported by Tang *et al.* (1994) that sterility inducing condition decreased the photochemical activity and efficiency of light energy transformation in PS II and markedly reduced the electron transmission of chloroplast in TGMS lines. This might be the cause for lower ATP and NADPH in the leaves of long day with high temperature treated sterile PGMS lines observed by Xia *et al.* (1993). Reduction in ATP content and carboxylation rate could be associated with impaired photochemical efficiency or excitation energy transfer to the reaction centre protein complex. The decrease in photochemical activity during high temperature might have decreased the concentration of photosynthetic products and affected male sterility as proposed by Tang *et al.* (1994). The result of the present investigation on hill reaction is also in conformity with the results of Roystephen (1998) and Ma Dehua *et al.* (1999).

5.3.1.3. Ethylene release rate

Ethylene is a gaseous hormone that synthesised in more amounts during stress conditions in plants. In the present experiment, the ethylene release rate of TGMS lines under fertile and sterile conditions was estimated by Gas chromatography techniques. The results showed an appreciable difference between the panicles of fertile and sterile TGMS lines in ethylene release rate. So it can be used as a potential parameter for identifying the TGMS character in rice genotypes. The ethylene release rate was negatively correlated with pollen fertility and was low in long day with low temperature and high under short day with high temperature (Li DeHong *et al.*, 1996). This was in accordance with the results of the present investigation. In the present investigation, all the fertile TGMS lines showed lesser concentration of ethylene compared to sterile lines in young panicles. This was in confirmity with the results of Luo *et al.* (1993). Treatment with amino ethoxyvinyl glycine (AVG), an inhibitor of ethylene biosynthesis, resulted in a marked increase in pollen fertility in male sterile lines. This indicated the key role of ethylene in the process of pollen sterility (Li DeHong *et al.*, 1996; Tian ChangEn, 2000). In the present study the release of more ethylene from sterile panicles of TGMS lines under high temperature and less ethylene from fertile panicles and normal variety under low temperature revealed the role of ethylene in fertility alteration under high temperature condition. This result was in confirmation with the findings of earlier studies by Zhu and Cao (1997) and Tian *et al.* (1999) in CMS lines.

5.3.1.4. Nutrient status

The content of secondary nutrient calcium (Ca) and the micronutrients copper (Cu) and boron (B), which play major role in reproductive growth of plants, were lower in leaves and young panicles of sterile TGMS lines compared to fertile condition. Graham, (1975) and Agarwala *et al.* (1979) found that boron and copper deficiencies induced male sterility in many cereals. They indicated that the deficiency of these

nutrients exhibited the reduction in anther size and pollen abortion. The pollen grains are poorly developed, small, shrivelled, and highly vacuolated and lack of proper exine and cytoplasmic contents. The reduction in boron content in sterile lines in the present study was in accordance with the results of Kaul (1988) that the pollen structure development and viability were highly affected by boron deficiency. Dell (1981) opined that the reduction in copper content cause minute anthers with scanty, dented, small, starchless abortive pollen grains confirming the results of present investigation. Wu and Li (1993) and Tian *et al.* (1998) suggested the importance of calcium in leaves and young panicles in determining pollen fertility. In sterile anthers, calcium precipitates were less in anther walls, pollen grain surfaces and these anomalies in the distribution of calcium accumulation caused the failure of pollen development and pollen abortion. From the present study, also it is evident that the deficiency of these nutrients causes failure of pollen development or pollen abortion, which leads to male sterility in TGMS lines.

5.3.2. Biochemical changes

5.3.2.1. Soluble protein

Protein, the immediate product of gene expression following transcription and translation, was the first nitrogenous compound lost under any stress situation (Martignone *et al.*, 1987). In the present study, soluble protein content reduced upto 40 per cent in leaves and young panicles under sterile condition whereas the content was gradually increased under fertile condition. This result was in confirmation with results proposed by Huang *et al.* (1994) in TGMS lines. Liu *et al.* (1993b) also reported a reduction in protein content under long days in PGMS lines Wang and Weicheng (1996) and Huang *et al.* (1996) suggested that reduced protein content in male sterile anthers and panicles under high temperature was also a cause for male sterility in TGMS lines. Kern and Atkins (1972) observed a reduction in the amounts of various amino acids in rice under sterile condition. This might be also the reason for decreased protein synthesis.

Roystephen and Thangaraj (2000) and Lakshmi Praba and Thangaraj (2000) also reported similar results as observed in the present study in sterile TGMS lines.

5.3.2.2. Carbohydrate fractions

5.3.2.2.1. Total carbohydrates

Analysing carbohydrate metabolism will be the best method for detection of any change in gene expression. Wang *et al.* (1991a) reported that the total carbohydrate content at flowering stage will be higher due to increase in rate of photosynthesis and regulation of source sink relation for better assimilate transport.

The present study revealed a reduction of total carbohydrates in leaves and young panicles under sterile condition in TGMS lines. Once amino acids are altered, a disturbed carbohydrate metabolism occurred in the male sterile lines of rice (Kaul, 1988). He *et al.* (1993) also reported the reduced carbohydrate content at every developmental stage of young panicles and leaves under sterile condition in PGMS lines. Loseva *et al.* (1974) proposed that the reduced activities of dehydrogenase and glucose-6-phosphate dehydrogenase enzymes, were the cause for reduction in carbohydrates under sterile condition, which led to a lower ATP content in male sterile anthers. Fertile TGMS lines and normal variety (ADT 36), exhibited continuous increase in the carbohydrate content upto flowering. Roystephen and Thangaraj (2000) also reported the accumulation of total carbohydrates in TGMS lines under fertile condition. From the earlier and present studies, it may be inferred that the reduced availability of hexose phosphate for the synthesis of complex carbohydrates was due to the reduced rate of photosynthesis and reduced supply of assimilatory powers.

5.3.2.2. Starch

Carbon assimilated during photosynthesis in the chloroplasts is stored temporarily as starch when if the triose phosphates are not translocated to cytosol for sucrose synthesis. The starch to sugar interconversion and the sugar transport are one of the basic factors deciding the development of panicles. Starch accumulation was more in leaves and less in young panicles under sterile condition in TGMS lines. Similar results were reported by Peng and Wang (1991) that an increase in starch content in leaves and decreases in young spikelets under sterility. The accumulation of starch in the chloroplasts might cause a reduction in photosynthesis mainly by inhibiting the light transmission to the lamellar system besides end product inhibition mechanism. The accumulation of starch in leaves revealed a reduced transport of sugars to the anthers and thus could be a cause for male sterility. This corroborated the results of present study in TGMS lines. Laskhmi Praba and Thangaraj (2000) reported a continuous accumulation of starch in leaves of TGMS lines under sterile condition. Wang *et al.* (1991a) also reported similar results in leaves but in spikelets accumulation was less in PGMS lines. In the present investigation also the accumulation of starch followed similar trend.

5.3.2.3. Total free amino acids

Amino acids are the building blocks of proteins and they reflect the physiological efficiency of plants. Characteristic differences in bound and free amino acids have been detected between male sterile and male fertile plants. In TGMS lines, an appreciable reduction of free amino acid content was noticed in leaves (49%) and young panicles (39%) under sterile condition. However, in fertile lines free amino acid content showed reverse trend. Kaul (1988) and Peng and Wang (1991) found a similar decrease in amino acid content in male sterile lines. The active nitrogen assimilation and normal protein synthesis were the causes for continued increase of free amino acid pool in fertile TGMS lines. This was in confirmation with the results of present investigation. Present results

also corroborates the report of Wang *et al.* (1993) that the free amino acid content reduced under sterility starting from the secondary branch primordia differentiation stage in PGMS lines. The reduction in free amino acid content was due to the reduction in the contents of leucine, isoleucine, phenylalanine and valine in the sterile anthers was reported in cereal crops like barley (Whited, 1967) and sorghum (Kern and Atkins, 1972). Kaul (1988) reported that due to the alteration in amino acids content, different or defective proteins are formed and a decreased or disturbed protein metabolism occurred. The reduction in free amino acids due to reduced nitrogen assimilation and disturbed protein synthesis might be the cause for male sterility.

5.3.2.4. Proline

In general, Proline content in plants could be considered as an index of stress resistance (Tang, 1984). Proline accumulates in the cytosol as a second line of defence by plants for maintaining the water potential during stress situation and also for maintaining the integrity of cellular organelles and functions in addition to its involvement in many physiological functions. Analysis of proline content as an index of male sterility, proposing a role for proline in causing male sterility of higher plants was reported by Luo *et al.* (1998). In the present study, there was 3-5 fold increase in proline content in leaves and young panicles of TGMS lines under sterile condition. Elsy (1997) and Lakshmi Praba and Thangaraj (2000) also reported similar results in the TGMS rice lines. Khan (1973) opined that possible accumulation of proline and hydroxy proline led to male sterility in rice. The accumulation may be either due to their increased synthesis or due to non-utilization of the same. But contradictory results were reported by Ozaki and Tai (1961) and Kaul (1988) who detected a decreased or complete absence of proline content in male sterile anthers. From the earlier and the present results it can be concluded that the accumulation of proline in leaves and spikelets has a definite role in affecting male sterility in TGMS lines. Proline may be acting as a signal molecule for

triggering other biochemical processes, which led to pollen abortion. This conclusion was drawn based on the fact that in the present investigation, proline accumulation was three fold at 20 DBH which coincided with the stamen and pistil differentiation which is the most critical stage of panicle development. Xiao *et al.* (1988) reported that the abnormal metabolism of free proline in the developmental process of the pollen in long day condition caused anther abortion in HPGMR lines. Xiao *et al.* (1993) opined that proline, as a kind of storage form, was able to co-ordinate with carbohydrates and the accumulation resulted in the inhibited synthesis of carbohydrates and else amino acids, which would have caused the abnormal development of pollens under sterile condition.

5.3.2.5. Total phenols

Phenols present in all plant species in the plant kingdom and they occur in all parts of plants. The phenols are highly influenced by change in environmental conditions and are associated with a wide range of growth and development processes in higher plants. Present investigation revealed the higher accumulation of phenols in leaves and young panicles of TGMS lines under sterile condition. Lakshmi Praba and Thangaraj (2000) have also reported higher accumulation of phenols in TGMS lines under sterile condition. Delalonde *et al.* (1996) suggested a negative correlation between phenolic content in anther and androgenic capacity. The content of phenols will be usually regulated by poly phenol oxidase (PPO) activity. Rodionova *et al.* (1995) found an association between phenolic compounds and activity of PPO in leaves. There was an accumulation of phenols and low activity of PPO under long day with high temperature but under short day conditions, reduced phenol content was noticed due to increased PPO activity. Roystephen (1998) opined that phenol accumulation can be one of the causes for male sterility since some phenols act as co-factors of IAA oxidase enzyme which was responsible for reduced IAA content under sterile TGMS lines. The present investigation

also showed an accumulation of phenols and enhanced activity of IAA oxidase which caused reduced quantities of functional auxin in high temperature induced sterility.

5.3.2.6. Ascorbic acid

Antioxidant systems of plants include non-enzymatic components such as ascorbic acid and glutathione. Both involved in active oxygen scavenging system as ascorbate peroxidase-glutathione reductase. Ascorbic acid content reduced from primary branch differentiation of panicle development until flowering in sterile TGMS lines, whereas in fertile TGMS lines and in normal variety it increased during that period. Liang *et al.* (1995) also reported an increase in ascorbic acid and glutathione content in leaves and anthers during fertile phase of PGMS lines. This corroborated the results of present study. Roystephen and Thangaraj (2000) inferred from their study in TGMS lines, that the reduced ascorbic acid content under sterility might have caused an accumulation of free radicals which in turn were responsible for causing pollen abortion. The results reported by Lakshmi Praba (1998) also confirmed the results of the present investigation.

5.3.2.7. Nucleic acids (DNA and RNA)

The specific changes in protein and enzymes would have resulted due to impaired metabolism of nucleic acids. Saini and Davis (1969) reported a reduction in the level of nucleic acids because of the low level of synthesis, probably due to malfunction of the enzyme systems, precursors or nucleotides required for nucleic acid synthesis.

In the present study, there was upto 35 and 37 per cent reduction in DNA and RNA content respectively in sterile TGMS lines. The contents increased gradually in fertile lines and in ADT 36 over stages, which showed an active metabolic process leading to flowering. Kaul (1988) also reported a reduction of nucleic acids in peas and rice mutants under sterile condition and the reduction were upto 54 per cent. Yang and

Zhu (1990) reported that there was 6.3-100 per cent reduction of total RNA content in PGMS lines under sterile condition. Kaul (1988) opined that this nucleic acid starvation at the beginning of meiosis caused meiotic arrest and meiocyte degeneration, which led to microspore abortion and thus male sterility. Lu *et al.* (1993) observed an association between male sterility and lower nucleic acids contents under long day condition. Chauhan and Kinoshita (1980) found that the injection of nucleic acids in onion caused reduction in pollen sterility which confirmed the role of reduced nucleic acids content in causing male sterility. Shu and Chen (1999) also reported that the DNA and RNA contents reduced or declined gradually in sterile TGMS lines and increased continuously in fertile lines as observed in the present experiment. The results of RNA content in the present study was in accordance with the results of Lakshmi Praba and Thangaraj (2000).

5.3.2.8. IAA oxidase (IAAO)

During reproductive phase of any crop plants, the activity of IAAO generally declines, so that the available auxin content can be utilized for 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 lines. In the present study, the IAAO activity increased under sterile condition upto 35 per cent in leaves and 51 per cent in young panicles compared to fertile lines. Similar results were also reported by Roystephen and Thangaraj (2000) that the higher activity of IAAO depleted the auxin upto 33 per cent in sterile TGMS lines. Shen and Gao (1992) opined that deletion of IAA might be one of the reasons for male sterility in PGMS lines. Increase in IAAO activity under sterile condition was also reported by the He and Xiao (1993) in TGMS lines. This increased enzyme activity cause deletion of IAA, which led to pollen abortion. In normal variety and fertile TGMS lines, reduction in the activity of enzyme made available more auxin in the reproductive stage. The increase was from primary and secondary rachis branch primordia differentiation of

panicle development. A difference between IAAO in fertile and sterile condition during the differentiation of stamen and pistil primordia observed by He and Xiao (1993), which corroborated the findings of the present study. The increase in IAAO activity of TGMS lines under sterile condition found in the present experiment led to deletion of IAA content which was associated with male sterility in TGMS lines by causing pollen abortion under high temperature (Lakshmi Praba and Thangaraj, 2000).

5.3.2.9. Nitrate reductase (NRase)

NRase is highly sensitive to environmental fluctuations. It is the important enzyme of nitrogen metabolism and involved in the conversion of nitrate to nitrite using assimilatory powers from chloroplasts. Jzochanjong and Chenchuang Jann (1982) related the higher NRase activity with flowering in higher plants. In the present investigation, NRase activity reduced upto 48 per cent in leaves and 45 per cent in spikelets under sterile condition in TGMS lines. This was in accordance with the results of Roystephen and Thangaraj (2000). They reported that the reduction in NRase activity reduced the nitrogen assimilation rate and synthesis of amino acids, thus acted as a cause for reduced free amino acid content in cytosol during sterility in TGMS lines, as observed in the present experiment. The results of Shen and Gao (1992) and Lakshmi Praba and Thangaraj (2000) were also in confirmation with the results of present study in TGMS lines.

5.3.2.10. Ascorbic acid oxidase (AAO)

The activity of AAO also varies in response to environment. The regulation of levels of oxidised and reduced glutathione, NADPH and ascorbate content are the major role of AAO in plants. AAO activity increased upto 2 fold in leaves and spikelets of sterile TGMS lines compared to fertile lines. Zhang *et al.* (1994b) reported similar results in AAO and glutathione reductase activities in PTGMS rice. Lakshmi Praba and

Thangaraj (2000) found that the activity of AAO increased due to induction of critical temperature during thermosensitive stage in sterile TGMS lines. The present study also revealed the constant reduction of AAO activity in fertile lines 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. Higher AAO activity under sterility might be the cause of reduced ascorbate content under sterile condition and thus associated directly with pollen sterility (Roystephen, 1998).

5.3.2.11. Glutamate dehydrogenase (GDH)

Glutamate dehydrogenase (GDH) occurs in almost all living organisms. In higher plants it existed in chloroplast and mitochondria as two different forms. In the present study, the GDH activity reduced in leaves and spikelets of TGMS lines under sterile condition during panicle development stages. But in fertile condition, the activity gradually increased in TGMS lines. Tang *et al.* (1994) opined that the reduction in GDH activity was due to a marked decline of photochemical activity of chloroplasts such as lower PSII activity leading to the impaired NADP reduction specifically required for the activity of GDH present in the chloroplast. Zhu and Yang (1992) found that the reduction in activities of dehydrogenases (ADH and GDH) affected the fertility percentage under long day with high temperature condition. This result was in accordance with the present investigation.

5.3.2.12. Malate dehydrogenase (MDH)

Malate dehydrogenase (MDH) is one of the enzymes involved in TCA cycle. MDH activity reduced under sterile condition in TGMS lines upto 32 per cent in leaves and young panicles. But the activity increased gradually in fertile lines and in normal variety. Zhang *et al.* (1994b) reported the same results in male sterile lines of PTGMS rice. LiPeng *et al.* (1997) found a change in MDH activity in TGMS lines during PMC

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formation, reduction division and pollen ripening stages and suggested that the abortion of pollen grains might be related to the decrease in MDH activity in spikelets. The present investigation was also in confirmation with these results.

5.3.2.13. Acid phosphatase (AP)

Acid phosphatase involved in the liberation of inorganic phosphate from organic phosphate esters. In TGMS lines the activity of acid phosphatase was reduced significantly in both leaves and young panicles under high temperature induced sterile condition. But in fertile lines there was no such reduction in acid phosphatase activity. This was in accordance with the results reported by Wang and Weicheng (1996) that the acid phosphatase activity was decreased during sterile condition compared to fertile condition in TGMS lines. Chen and Zhou (1997) also reported similar results in PTGMS lines during high temperature with long days. LiPeng *et al.* (1997) found the change in activity of acid phosphatase in TGMS lines during PMC formation, reduction division and pollen ripening stages and opined that the abortion of pollen grains was closely related to the decrease in acid phosphatase activity in spikelets. This result was in accordance with the results of the present investigation.

5.3.2.14. Catalase (CAT)

Catalase (CAT) is mainly involved in the destruction of hydrogen peroxide and oxidation of hydrogen donors. The enhanced production of most reactive hydroxyl radicals, which cause peroxidation of unsaturated lipids of cell membrane and damaging the structural integrity of the membranes, change the structure and functions of proteins including enzymes and nucleic acids is due to impaired activity of catalase. Catalase activity was reduced in sterile TGMS lines under high temperature. The reduction in the activity was upto 65 per cent in leaves and 49 per cent in young spikelets compared to fertile condition during different stages of panicle development. Zhu and Yang (1992)

reported that the reduction in CAT activity was one of the causes for reduction in fertility percentage. Raj and Siddiq (1986) and Liang and Chen (1993) opined that the weaker oxygen scavenger system and a high level of lipid peroxidation in anthers were responsible for male sterility in CMS and T(P)GMS lines. Shen and Cao (1992), Zou *et al.* (1993) and Zhang *et al.* (1994b) also reported a reduction of CAT activity in sterile EGMS lines. The findings of the present experiment confirmed the results of Roystephen and Thangaraj (2000) who reported the role of active oxygen species in causing pollen abortion in TGMS lines. They observed a reduction in photochemical efficiency and disturbance in protein and nucleic acids metabolism besides lower enzyme activities due to lower CAT activity under sterile condition. Similar reasons were also suggested by Zou *et al.* (1993) for pollen sterility in PGMS lines.

5.3.2.15. Peroxidase (POX)

Peroxidase is an important enzyme involved in morphogenesis and auxin oxidation. It is the enzyme, which is very sensitive to environmental fluctuations and is considered as the measure of plant's resistance to stress. The major function of POX is the catalytic conversion of H_2O_2 produced by SOD to water. In sterile TGMS lines, there was upto 37 per cent reduction of POX activity in leaves and young panicles compared to fertile TGMS lines. The reduction in POX activity was marked even at the beginning of secondary branch primordia differentiation. These results were in confirmation with the results of Chen and Xiao (1987) in PTGMS rice, Cheng and Xiao (1993) in PGMS rice. He and Xiao (1993), Zhang *et al.* (1994b) and Zhu and Cao (1997) also confirmed the increased POX activity under fertile condition during sensitive stages. Chen and Zhou (1997) found a decreased activity of POX in PTGMS lines under long days with high temperature. Roystephen and Thangaraj (2000) reported the similar results in TGMS lines under high temperature and opined that the abnormal activity of POX might be associated with male sterility by strengthening the role of active oxygen species which

cause pollen abortion in TGMS lines. The results of the present experiment were in confirmation with the earlier findings.

5.3.2.16. Superoxide dismutase (SOD)

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. In the present study, the SOD activity reduced drastically during CST treatment at different stages of panicle development in TGMS lines. Shen and Cao (1992) observed the same results in sterile PTGMS lines under high temperature with long days. He and Xiao (1993) suggested that the change in SOD activity was closely related with the transformation of fertility in TGMS lines. Zhang *et al.* (1994b) also reported higher activity of SOD in fertile phase than in sterile phase of TGMS panicles. The results of the present experiment was in accordance with the findings of Roystephen (1998) who suggested that higher temperature was an adversity for TGMS lines where it produced more superoxide radicals causing membrane damage and this might be one of the most likely reasons 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.

5.3.3. Molecular mechanisms of TGMS

5.3.3.1. Study of protein profile in TGMS lines under sterile and fertile conditions

The results of SDS-PAGE protein profile of TGMS lines showed that there existed quantitative difference in protein profile between fertile and sterile conditions. The leaf samples of TGMS lines showed an enhanced expression of 64, 54 and 24 kDa proteins under fertile condition and in anthers 48, 38 and 22 kDa proteins expressed in higher intensity under fertile condition. Under sterile condition, the above said proteins

were expressed in lower intensity in both the samples, but a smaller molecular mass protein of 14 kDa was expressed only in anthers under sterile condition. The lower intensity in the expression pattern of 54 kDa protein profiles in leaves of TGMS lines under sterile condition was due to impaired synthesis of larger subunit complex of Rubisco under stress (Hidema *et al.*, 1992). The lower expression of 54 kDa protein under sterile condition was also confirmed by the results of Miller (1988) who reported the quantitative change in Rubisco under high temperature condition, since it was coded by chloroplast genome. High temperature stress caused membrane damage, protein denaturation and mutation of DNA (Bowler *et al.*, 1992). This might be also the reason for lower expression of other proteins at 64 and 24 kDa in sterile condition. The enhanced expression of proteins in leaves of TGMS lines at molecular weight of 64 and 24 kDa was seen in fertile plants. This was in accordance with the findings of Roystephen and Thangaraj (2000). The enhanced expression of 48, 38 and 24 kDa proteins in fertile TGMS lines suggested their role in fertility expression in TGMS lines and the presence of smaller molecular weight protein (14 kDa) might be associated with the expression of male sterility genes in TGMS lines. The characterisation of protein which expressed under sterile condition will be useful to understand the expression of male sterility genes in TGMS lines. Huang *et al.* (1994) reported the differences in protein components of anthers of PGMS lines under fertile and sterile conditions. They have observed two specific bands at 43 and 40 kDa in fertile anthers. However, in the present study TGMS lines expressed two high intensity protein bands at around 48 and 38 kDa for fertile anthers. These findings confirmed the reports of LimeiRu *et al.* (1999) that the proteins at the isoelectric focus point was associated with fertility expression during panicle development. This result suggested the possible role of these proteins in fertility and sterility expression in TGMS lines.

5.3.3.2. Isozyme analysis

In the present investigation, isozyme patterns of TGMS lines were analysed under fertile and sterile conditions in comparison with a normal variety (ADT 36) to detect the polymorphism. Li *et al.* (1994) have reported that isozymes have a definite role on regulating plant growth and development. Freeling (1983) observed that isozyme patterns were the better biochemical means to analyse the interactions with higher accuracy. The focus of present investigation is to understand the mechanisms of TGMS in rice at biochemical level by the antioxidant enzymes like catalase, peroxidase and superoxide dismutase. Besides these enzymes, esterase and polyphenol oxidase also play a crucial role in the regulation of fertility/sterility in TGMS lines but, which needs further confirmation.

5.3.3.2.1. Free radical scavenging enzymes

The polymorphism analysis of catalase showed the occurrence of different isoforms under fertility and sterility in TGMS lines and in normal variety. The number of isoforms was more (CAT 1, 2 and 3) in fertile TGMS lines and in normal variety. But sterile genotypes produced only two bands (CAT 1 and 2). The CAT 3 might be associated with fertility expression since it appeared only in fertile lines and normal variety. The intensity of isoforms also more under fertile condition. This was in line with the results of Tong *et al.* (1993) who reported the decreased activity, intensity and number of bands of catalase enzyme in sterile TGMS lines. The impaired activity and low level of expression of catalase will enhance the production of reactive oxygen species, which cause peroxidation of membrane lipids and lead to abortion of pollen grains (Shen and Gao, 1992).

The analysis of isozyme pattern of peroxidase revealed the presence of specific isoforms under fertile and sterile conditions in TGMS lines. Liu *et al.* (1993a) reported

that the isozyme band number and intensity were different under fertile and sterile conditions. Li *et al.* (1993) also observed the difference in peroxidase isozyme banding pattern of fertile and sterile PGMS lines with two extra bands under fertile condition. In the present experiment, also there were four extra isoforms in TGMS lines under fertile condition. The POX 4 and 5 were associated with fertility of TGMS lines which were also present in the normal variety ADT 36, but were absent in sterile TGMS lines. Wu and Xiao (1993) found variation in peroxidase isozyme banding pattern and its activity in TGMS lines. A specific band POX 1 was present only in TGMS lines with uniform high activity. Elsy (1997) reported a similar isozyme banding pattern of peroxidase under high and low temperature except the presence of POX 23 under high temperature. In this experiment POX 6 and 7 were present in all the TGMS lines under fertile and sterile conditions. This showed the similarity between the TGMS lines under fertile and sterile conditions. The results of present investigation confirmed the earlier results by Lakshmi Praba and Thangaraj (2000).

The isoenzymatic pattern of superoxide dismutase showed the presence of more number of bands (5 bands) under fertile condition than under sterile condition (3 bands) in TGMS lines. Fertile TGMS lines yielded two extra bands regardless of genotypes and the intensity of the bands were more compared to the sterile lines. The results of the present study was in support of the findings of Zhang *et al.* (1994b) and Roystephan (1998) who observed higher SOD activity under fertile condition compared to sterility in PTGMS lines. From the findings of the present experiment, it could be inferred that for TGMS lines, high temperature was an adversity which produced more reactive oxygen species causing membrane damage, change in structure and function of DNA and RNA and denaturation of proteins. This might be the cause for the absence of certain isozymes and the lesser intensity of isoforms present under sterile condition.

3.3.3.2.2. Esterase (EST)

Esterase isozyme analysis showed the presence of different isoforms in various TGMS lines under fertile and sterile conditions. From the Plate 8 it was evident that EST 1 and 3 might be well associated with fertility expression, since it appeared only under fertile condition in TGMS lines and also in normal variety ADT 36. Hu and Wan (1991) and Li *et al.* (1994) reported the relationship of a specific esterase isozymes with male sterility in rice. This was in line with the results of Li *et al.* (1995). The lower intensity of EST isoforms in sterile condition implied their role in sterility expression in TGMS lines as in the present study confirmed the findings suggested by Roystephen and Thangaraj (2000).

5.3.3.2.3. Polyphenol oxidase (PPO)

Polyphenol oxidase isozyme pattern showed appreciable discrimination in banding pattern between fertile and sterile genotypes. Like esterase, this also showed a specific banding pattern to fertile TGMS lines. The presence of PPO3 in fertile TGMS lines was associated with fertility expression under fertile condition. Its presence in normal variety confirmed its role in fertility expression. The PPO 1 and 2 were present in all the TGMS lines in both the conditions revealed their association with TGMS in rice. But their intensity decided the fertility or sterility of TGMS lines. So from this study, it can be concluded that specific banding pattern may be taken as biochemical marker for identifying sterile and fertile genotypes.

Interestingly, the isozyme banding patterns of these enzymes showed distinct difference in isoforms between TGMS lines and normal variety. This indicated the presence of specific association between TGMS lines alone and suggested this as a biochemical marker for screening TGMS lines in rice.

However, the above discussed enzymes need further investigation to have concrete idea.

5.4. Morphological and anatomical changes of anthers associated with male sterility in TGMS lines

In the present study, morphological and anatomical examination of both male fertile and male sterile anthers of TGMS lines revealed the abnormal development of anthers with reduced pollen size under sterile condition. There were distinct differences in the development of anther wall and tapetum under sterile and fertile conditions. In the sterile anthers of genic male sterile rices, Cheng *et al.* (1982) observed high degree of tapetal anomaly. Feng *et al.* (2000) suggested that the abnormalities of microsporocytic cytoplasm or pollen exine rather than the tapetal development might be the reason for pollen abortion in PTGMS rice. Prasad and Rao (1996) also observed the differences in anther sac layers of CGMS line of rice. The fertile anthers had round, oval and larger pollen grains whereas sterile pollen grains were deformed, sickle shaped and crumbled in appearance. This was in conformity with the observations of anthers and pollen grains under light microscope in the present study. Similar results were reported in rice by Chaudhuary *et al.* (1981) and Sun and Zhu (1995).

In the present study, the microtomy sections of male sterile anthers showed persistent tapetum whereas in male fertile anthers tapetum degenerated with thickened endothecium and epidermal cells. These observations were in confirmation with the findings of Lakshmi Praba (1998) in TGMS lines. Observations on the changes in anther microstructure of PGMS rice under long day condition by Wang *et al.* (1992) showed the abnormal and poor development of vessel elements in sieve tubes in vascular bundles of anther septa and wrinkled abnormal shaped sheath cells and also the anther cytoplasm of tapetal cells without degeneration under long day treated plants. From the results,

Wang *et al.* (1992) suggested that the abnormal alterations in the vascular bundles and tapetum tissue might have reduced nutrient supply to the developing microspores in anthers under high temperature causing sterility.

5.5. Chemical manipulation of fertility / sterility in TGMS lines

5.5.1. Induction of sterility by chemicals and plant growth regulators (PGRs)

When sprayed on rice plants at specific developmental stages, a chemical or plant growth regulator can emasculate the plants, thus resulting in male sterility while maintaining normal female fertility (Lasa and Bosemark, 1993). Obtaining stable male sterility by a simple chemical treatment without any accompanying adverse effect on female fertility have many advantages in two line breeding using TGMS system, since the temperature may fluctuate below CST during thermosensitive stage making the line only partially sterile. This reduces quality of seeds since selfed seeds also will be there along with hybrid seeds (Ali, 1996). In the present study, certain chemicals and growth regulators were tried to get complete male sterility in TGMS lines. Among the treatments with different concentrations, Salicylic acid 800 ppm, MH 0.1 per cent and ethrel 400 ppm induced more than 99 per cent spikelet sterility in all the TGMS lines.

In the present investigation total phenols were found to accumulate in the leaves and young panicles under high temperature induced sterility. The exogenous application of salicylic acid also increased the leaf proline, total phenols content and IAA oxidase activity while inducing male sterility in TGMS lines. Similar results were observed by Thangaraj and Lakshmi Praba (2000) due to salicylic acid spray in TGMS lines. Luo *et al.* (1998) suggested that, analysis of proline content could be used as an index of male sterility, proposing a role for proline in causing male sterility in higher plants. Proline may be acting as a signal molecule for triggering other biochemical processes which led to pollen abortion. Roystephen and Thangaraj (2000) also reported that the

depletion of IAA due to higher activity of IAA oxidase was one of the major causes for male sterility in TGMS lines. This was in accordance with results of Khan (1973) in rice. Raskin *et al.* (1987) observed that application of salicylic acid at 0.13 µg per gram fresh weight of sections of the immature spadix of *Arum* lillies led to temperature increase of as much as 14°C above the ambient temperature. In the present study, the male sterility in TGMS lines induced by salicylic acid spray, might be due to the increase in temperature above the CST around the reproductive part after the treatment. Salicylic acid induced thermogenesis may trigger the expression of the gene responsible for male sterility in TGMS lines causing the pollen abortion and sterility. Thangaraj and Lakshmi Praba (2000) reported that the salicylic acid (800 ppm) spray at panicle initiation stage induced complete pollen sterility in TGMS lines. This induced sterility was found to be associated with an increased activity of IAA oxidase and accumulation of phenols which caused thermogenesis in TGMS lines would be the possible reason for pollen sterility. This was also confirmed by Roystephen and Thangaraj (2000). They reported that salicylic acid, being a phenolic acid induced more than 90 per cent male sterility in TGMS lines. Delalonde *et al.* (1996) reported a correlation between phenolics in anthers and androgenic capacity.

Ethrel 400 ppm spray induced 99 per cent pollen sterility in TGMS lines. Aswathanarayana and Mahadevappa (1991) also reported the induction of male sterility by ethrel in rice. Similar results were also reported by Rowell and Miller (1971) and Ladislav and Marie (1978) in wheat, Thakur and Rao (1988) in pearl millet and Liang *et al.* (1999) in rice. Luo *et al.* (1993) observed a higher ethylene release rate under sterility in young panicles of TGMS lines which was negatively correlated with pollen fertility. The ethylene released by the compound ethrel would have caused male sterility in TGMS lines. Thangaraj and Lakshmi Praba (2000) found that foliar spray of ethrel

(400 ppm) at panicle initiation stage caused more than 90 per cent pollen sterility in TGMS lines.

Maleic hydrazide at 0.1 per cent also induced (99%) pollen sterility in TGMS lines. The results of Roystephen (1998) in TGMS lines, Singh (1964) in cotton, Kaul and Singh (1967) in *Phaseolus*, Chopra *et al.* (1960) in wheat, Aswathanarayana and Mahadevappa (1992) in rice and Natrova (1973) in rye were also revealed the efficiency of MH in inducing male sterility. The exogenous application of GA₃ restored the fertility of Nongken 58S plants (Tong *et al.*, 1993). MH being an antigibberillin could have reduced the GA content and caused pollen abortion. Manjula and Ibrahim (1999) reported that MH spray caused more than 90 per cent pollen sterility, and anther deformation and thus high spikelet sterility. In the present investigation among the different stages of spray, PI alone, PI and 5 DA PMC and PI + PMC + 5DA PMC caused more than 99 per cent spikelet sterility in TGMS lines for salicylic acid at 800 ppm. Among these three stages of treatment, salicylic acid sprayed at PI alone was selected as the best stage for inducing complete pollen / spikelet sterility because a single spray of salicylic acid at PI stage caused complete pollen sterility in TGMS lines compared to the other 2 stages of treatment which caused complete sterility with combination of sprays. Similar results were obtained by Parmer *et al.* (1979) who reported that stages between midprimordia and boot leaf were most effective for the induction of male sterility in rice.

In view of the encouraging results obtained from the present investigation, with the objective of screening suitable chemical or PGR for inducing complete pollen sterility besides contributing the most responsive phase of development that would ensure maximum pollen sterility with least phytotoxic and other undesirable effects of the chemicals, salicylic acid proved best among the chemicals or PGRs, used for inducing complete pollen sterility, at 800 ppm dosage level sprayed at PI stage. Though ethrel

400 ppm and MH 0.1 per cent also induced complete pollen sterility at certain stages of spray, salicylic acid (800 ppm) at PI stage was selected because it was not caused any phytotoxic effects and also no adverse effect on female fertility which was also proved in the present investigation. But ethrel and MH caused phytotoxic effects such as reduction in plant height, inhibition of culm elongation which caused incomplete exertion of panicles and scorching of panicles which led to affect the female fertility. This was in accordance with the results of Huges *et al.* (1978).

5.5.1.1. Effect of salicylic acid spray on pollen sterility and crossed seed setting

The aim of this present experiment was to evaluate the efficiency of salicylic acid for obtaining male sterile TGMS lines under low temperature conditions, which fulfil the following stringent criteria : Complete expression of male sterility, perfect female fertility and no phenotypic deviations from the parent of origin.

In the present experiment, the per cent pollen sterility indicated the effect of salicylic acid treatment on induction of male sterility in TGMS lines. The complete pollen sterility observed in TGMS lines in the present study confirmed the results of Thangaraj and Lakshmi Praba (2000). They observed complete pollen sterility due to salicylic acid treatment at PI stage in TGMS lines. This chemically induced male sterility was found to be associated with an increased activity of IAA oxidase which depleted the auxin content and accumulation of phenols which caused thermogenesis around the reproductive parts, would be a possible reason for pollen sterility. The absence of selfed seed setting indicated the complete abortion of pollen grains with no development of fertile anthers due to salicylic acid treatment. This was in accordance with the results of Manickam and Sarkar (1999) who reported the inhibitory effect of salicylic acid on pollen grains of maize plants.

In the present investigation, the per cent crossed seed setting in salicylic acid induced male sterile TGMS lines indicated the ability of ovule to produce seeds whenever there was a chance of cross pollination. The seed setting in the chemically induced male sterile spikelets revealed that the treatment of salicylic acid did not affect the female fertility, but caused only pollen sterility in TGMS lines. In the present study, the out crossing potential of salicylic acid induced sterile TGMS lines was proved by cross pollination with male parent. The crossed seed setting revealed the presence of viable, fertile ovary in the salicylic acid induced male sterile spikelets (Plates 14 and 15).

5.5.1.2. Biochemical changes associated with salicylic acid induced male sterility in TGMS lines

5.5.1.2.1. Soluble protein

In the present investigation, the influence of salicylic acid spray, while inducing male sterility in TGMS lines, showed a reduction in soluble protein content during different stages of panicle development. Similar results were reported by Pancheva and Popova (1998) that salicylic acid inhibited the synthesis of total soluble protein and more particularly the synthesis of Rubisco. Asthana and Srivastava (1977) suggested that the reduction in protein content was due to the reduction of protein N by salicylic acid treatment. Male sterility induced by pyridoxinone derivatives in rice was closely related to the deficiency of protein during anther development (Zhang *et al.*, 1996). This was in accordance with the findings of LimeiRu *et al.* (1999) in dual purpose genic male sterile lines. These results were in conformity with the results of present investigation. Mitra and Sen (1966) reported that inhibitors of protein synthesis and increased protease enzyme activity impaired the flowering process. Thus it was suggested that the inhibition of protein synthesis by treatment with salicylic acid was one of the causes for male sterility.

5.5.1.2.2. Carbohydrate fractions

The total carbohydrate content was found to be low in salicylic acid treated plants during sterility induction in TGMS lines. Liu *et al.* (1998) reported that the treatment of CHA "Baochungling" decreased the soluble carbohydrates in PTGMS lines. Janda *et al.* (1998) reported that decrease in the rate of photosynthesis due to salicylic acid treatment, decreased the assimilation of total carbohydrates in maize plants.

Xiao *et al.* (1996a) found that the CHA "Jinaolin" caused reduction in total carbohydrate content during pollen abortion in wheat. Shehata *et al.* (2000) reported that the salicylic acid spray decreased the total soluble sugars in cotton. These results were in accordance with the results of present study. The reduction in carbohydrate content might be coupled with reduced dehydrogenase and Glucose-6-phosphate dehydrogenase enzyme activities. This led to a lower ATP content. Reduction in ATP content indicated a low synthetic capacity and energy effectiveness of oxidative processes which in turn led to male sterility (Kaul, 1988).

The increase in starch content was noticed during panicle development stages, when the TGMS lines were treated with salicylic acid. This was in confirmation with the results of Rajendra and Bates (1981) in wheat. HaroldMcRae (1985) reported that the decreased starch deposition in anthers was due to the treatment of chemical hybridizing agents. It might be due to the poor conversion and translocation of starch from leaves to reproductive parts. This increase in starch content in leaves could be a basic cause of male sterility (Peng and Wang, 1991).

5.5.1.2.3. Nucleic acids (DNA and RNA)

The nucleic acids content was significantly reduced in salicylic acid induced male sterile TGMS lines during panicle development stages compared to untreated fertile lines.

In PTGMS lines, Liu *et al.* (1998) reported that treatment of chemical hybridizing agent 'BCL' caused reduction of RNA and DNA contents in leaves. Similar results were also reported by Tian *et al.* (1999) that the ethylene treatment markedly reduced the RNA and DNA contents in rice. Application of pyridoxinone derivatives decreased the total nucleic acids in rice variety Guangluluai4. This deficiency of nucleic acids was closely related with male sterility (Zhang *et al.*, 1996). These results were in confirmation with the results of present study. The reduced nucleic acids content might be due to malfunction of enzyme systems, precursors or nucleotides required for nucleic acid synthesis. Thus a low level of nucleic acids content in the leaves and associated inadequate transport to the developing anthers could be a limiting factor in the development of microspores to mature pollen grains.

5.5.1.2.4. Proline

Salicylic acid treatment induced 2-3 fold increase in accumulation of proline in leaves while inducing male sterility in TGMS lines. Thangaraj and Lakshmi Praba (2000) also reported accumulation of proline due to salicylic acid treatment in TGMS lines. Chemical hybridizing agent 'Jinaolin' treatment caused more than 98 per cent pollen sterility with increase in proline accumulation in wheat (Xiao *et al.*, 1996a). Khan (1973) suggested that the accumulation of proline might be either due to their increased synthesis or due to non utilization of the same.

5.5.1.2.5. Total phenols

In the present investigation, a greater accumulation of total phenols in the leaves of TGMS lines was observed due to salicylic acid treatment when compared to control plants. Similar results were reported by Thangaraj and Lakshmi Praba (2000). They suggested that male sterility induced by salicylic acid was found to be associated with an increase in accumulation of phenols which caused thermogenesis in TGMS lines.

This was in accordance with the results proposed by Delalonde *et al.* (1996). Roystephen and Thangaraj (2000) reported that phenol accumulation could be one of causes for male sterility since some phenols act as co-factors of IAA oxidase enzyme which is responsible for reduced IAA content under sterile condition. These results were in conformity with the results of present investigation.

5.5.1.2.6. IAA oxidase (IAAO)

Characteristic differences in the IAAO activity in the leaves of TGMS lines were observed between salicylic acid treated and control plants. An increase in activity of IAAO was noticed in TGMS lines during panicle development stages when the plants were treated with salicylic acid for the induction of male sterility. Similar results were observed by Tang *et al.* (1996) in rice plants. They reported that the male sterility induced by TO3, a chemical hybridizing agent, caused reduction in IAA due to increased activity of IAA oxidase. Thangaraj and Lakshmi Praba (2000) observed 100 per cent pollen sterility with salicylic acid treatment in TGMS lines. This chemically induced male sterility was found to be associated with an increased activity of IAA oxidase which caused deletion of IAA. This would be a possible reason for pollen sterility in TGMS lines. This also corroborated the results of the present study. Huberman *et al.* (1997) suggested that the reduction in IAA resulted in impaired auxin transport capacity which was the major mechanism by which chemical hybridizing agents caused abnormal reproductive organs.

5.5.1.2.7. Ascorbic acid oxidase (AAO)

The activity of enzyme AAO increased upto 60 per cent in TGMS lines during panicle development stage due to the application of salicylic acid compared to control. This many fold increase could be correlated with reduced ascorbic acid content. Roystephen and Thangaraj (2000) suggested that the higher activity of AAO might be the

cause for reduction in ascorbic acid content, which acted as a potent antioxidant in plants. The reduction in ascorbic acid content might cause an accumulation of free radicals which in turn were responsible for pollen abortion in TGMS lines.

5.5.1.2.8. Free radical scavenging enzymes

From the present investigation, distinct differences were observed in the activities of free radical scavenging enzymes, catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) in TGMS lines. Salicylic acid treatment caused up to 43.0, 21.6 and 26.0 per cent reduction in the activities of CAT, POX and SOD respectively during reproductive stage. Similar results were also observed by Tian *et al.* (1999) with ethrel treatment in rice. They reported that the treatment with ethrel markedly reduced the activities of enzymes CAT, POX and SOD. Chenzhi *et al.* (1997) have reported the inhibition of catalase activity by salicylic acid treatment in rice. This was in accordance with the results of Dat *et al.* (1998) who reported the reduced activities of CAT and POX in mustard plants due to salicylic acid treatment. Similar results were reported by Lopez *et al.* (1998) in potato plants with acetyl salicylic acid treatment. The reduction in the activity of these enzymes resulted in accumulation of H_2O_2 and O_2^- molecules which may restrain the development of pollen. The results of the present study also confirmed the earlier findings.

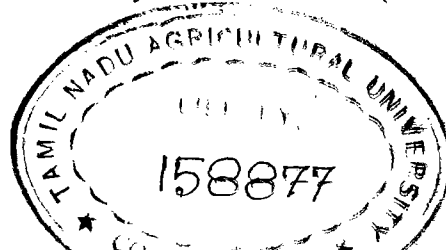
5.5.2. Induction of fertility by growth regulators

The concept of using chemicals to restore fertility in genetic male sterilities have been of interest as long as there has been interest in chemical hybridizing agents. Several scientists have drawn attention to the advantages of fertility restoration as compared with the selective male sterility approach (Laible, 1974; Foster, 1981). The advantages of chemical restoration of genetic male sterility are at least three fold. Firstly, the chemical need not be 100 per cent effective since it is used only to increase of female parent seed.

Secondly, all seed produced is exactly what is wanted - it is a failsafe method (Laible, 1974). Thirdly, since the chemical doesnot have to be used in the hybrid seed production field, a low volume is required compared with the amount of chemical hybridizing agent (CHA) required. This is inturn has favourable impact on crop and environment (Harold McRae, 1985).

In the present investigation different hormones and growth regulators were applied exogenously to study their effect on pollen fertility and seed setting and also to confirm the role in pollen development of TGMS lines. Among the treatments, IAA (50 ppm) performed well by increasing the seed setting percentage to 38.5. There is no report on the restoration of fertility by IAA spray in TGMS lines. Tong Zhe (1998) reported that gibberellines and auxin may be the chemical signals for fertility regulation. You *et al.* (1997) also suggested that the level of male sterility was related to the loss of endogenous hormones (especially IAA). IAA was highly associated with pollen development. In the present experiment also the deletion of IAA was found to be one of the major causes of male sterility in TGMS lines. This was in confirmation with the results of Shen and Gao (1992) and Thangaraj and Lakshmi Praba (2000).

Brassino steroids (BRs), a group of naturally occurring plant hormones initially isolated from pollen of *Brassica napus*, have a definite role in reproductive growth especially for the development of pollen (Grove *et al.*, 1979). In the present investigation BR at 1 ppm spray increased the seed set of TGMS lines upto 25.2 per cent. This was in confirmation with the results of Thirthalingappa (1999) who reported that the spray of BR at 0.1 ppm increased the percentage of seed set in CMS lines. This was also reported by Roystephen (1998) in TGMS lines. Braun and Wild (1984) and Sairam (1994) reported that the brassinosteriods helped the pollen development by increasing the nucleic acids and protein content besides the Rubisco activity. Kalinch *et al.* (1985)



reported an increase in RNA, DNA and protein contents in BR treated mungbeans and beans and they suggested that the involvement of BR in transcription and replication led to increase in enzyme activities during pollen development and tissue growth.

With the encouraging results from the present study in induction of male fertility and sterility in TGMS lines, it was concluded that the application of IAA (50 ppm) at panicle initiation stage was the best in inducing male sterility in TGMS lines. In the present experiment IAA (50 ppm) recorded 38.5 per cent seed set in TGMS lines. This result was in confirmation with the suggestions of Yuan (1994) and Ilyas Ahmed (1996). They proposed that more than 30 per cent of seed set during fertile phase of TGMS lines was effective in hybridization programme. Since TGMS lines have comparatively lesser seed setting, any attempt to increase the seed set will be very much useful for efficient seed multiplication in TGMS lines. From the results of the experiment on induction of male sterility by chemicals and growth regulators, it was concluded that the salicylic acid (800 ppm) treatment at panicle initiation stage was the best among all the treatments. This treatment will also be useful to induce complete male sterility during fluctuations of temperature. This treatment is cheaper and highly efficient when compared to other available methods. This would also ensure high quality hybrid seeds combined with increased productivity.

Summary

CHAPTER V

SUMMARY

Investigations were carried out with three TGMS lines *viz.*, TGMS 6, TGMS 16 and TGMS 29 and a normal variety ADT 36 to standardize the critical sterility temperature (CST) and critical stage of thermosensitivity using phytotron. The physiological, biochemical and molecular aspects were determined during different stages of panicle development in fertile and sterile TGMS lines to explain the exact cause for male sterility. Isozyme banding pattern of certain enzymes were carried out to show the polymorphism of enzymes under fertile and sterile conditions and to study their potential for utilizing as biochemical marker for TGMS lines. Morphological and anatomical changes in anthers and pollen grains were analysed under fertile and sterile conditions by light microscope and microtome sections. Based on the results, the possibility of chemical manipulation of fertility / sterility was also investigated using different chemicals and growth regulators. The physiological and biochemical changes associated with chemical induction of sterility was also studied. The results are summarised below.

- ♦ The critical sterility temperature differed with the TGMS lines. The CST of selected TGMS lines *viz.*, TGMS 6, TGMS 16 and TGMS 29 was daily mean temperature of 28.0, 27.0 and 27.5°C respectively.
- ♦ Irrespective of TGMS lines, the thermosensitive stage at which fertility transformation took place was 10 to 25 days before heading which coinciding with secondary branch primordial differentiation to early meiosis of pollen mother cell formation.

Physiological parameters

- ♦ Photosynthetic rate and hill reaction rate reduced under sterile condition which was due to reduction in Rubisco content, decrease in photochemical activity and light energy transformation in PS II under high temperature condition.
- ♦ Reduction in micronutrients (Ca, Cu and B) content was associated with male sterility. These nutrients played an important role in the development of reproductive parts.
- ♦ Increased ethylene release rate under sterility revealed its possible role in determining male sterility in TGMS lines.

Biochemical aspects

- ♦ Reduction in soluble protein under sterility was due to impaired synthesis of amino acids and nucleic acids in TGMS lines under high temperature condition.
- ♦ Reduction of total carbohydrates and accumulation of starch under high temperature induced sterility showed the impaired synthesis and translocation of carbohydrate fractions.
- ♦ Proline and phenols accumulated several folds under sterile condition. Proline might be acting as signal or messenger molecules to induce male sterility by the inhibition of carbohydrate and amino acid synthesis. Phenols acting as co-factors for IAA oxidase which depleted the IAA content.
- ♦ Ascorbic acid content decreased under sterility due to high temperature induced increase in ascorbic acid oxidase activity.
- ♦ IAA oxidase activity increased under sterile condition in TGMS lines. This was the main cause for the reduction of IAA content, which was associated with male sterility.

- ♦ The reduced activity of nitrate reductase under sterility revealed its association with reduction in amino acids, nucleic acids and protein contents.
- ♦ Reduction in the activity of enzymes *viz.*, GDH, MDH and acid phosphatase under high temperature condition caused abortion of pollen grains.
- ♦ High temperature induced reduction in the activity of free radical scavenging enzymes *viz.*, CAT, POX and SOD resulted the membrane damage due to the accumulation of active oxygen species.

Molecular aspects

- ♦ Electrophoretic study of protein profile in leaves and anthers showed a quantitative difference between fertility and sterility. There existed a reduction in the larger subunit of Rubisco and expression of smaller molecular weight protein under sterility.
- ♦ Isozyme banding pattern of CAT, POX, SOD, EST and PPO revealed the presence of distinct isoforms with polymorphic nature under fertile and sterile conditions. These isoforms showed distinct difference between TGMS lines and between TGMS lines and normal variety. The presence of specific isoforms only in TGMS lines showed the potential of using the isozyme pattern as efficient biochemical marker for screening TGMS trait in rice.

Morphological and anatomical changes

- ♦ Light microscope and microtome section studies showed the shrivelled, ill filled and malformed sterile anthers under high temperature treatment. Distinct differences were also exhibited by the fertile and sterile anthers with respect to anatomy.

Induction of fertility / sterility

- ♦ Certain chemicals and growth regulators were efficient in the induction of fertility/sterility. Salicylic acid (800 ppm) at PI stage was found to be the best alternative to ethrel and MH in inducing male sterility in TGMS lines.
- ♦ Increased crossed seed set percentage revealed no adverse effect on fertility by salicylic acid spray for the induction of male sterility in TGMS lines under unfavourable weather conditions.

Biochemical changes associated with induction of male sterility

- ♦ A reduction in soluble protein, total carbohydrates and nucleic acids content and increase in proline and total phenol contents was noticed in salicylic acid induced male sterile TGMS lines.
- ♦ Activities of antioxidant enzymes *viz.*, CAT, POX and SOD decreased and IAA oxidase and ascorbic acid oxidase activities increased. These changes revealed the mode and mechanism of action of salicylic acid in induction of male sterility in TGMS lines.
- ♦ IAA (50 ppm) and BR (1 ppm) increased the pollen fertility and seed set which confirms their role in reproductive parts development in rice.

From the findings of the present investigation, it is evident that the knowledge on physiological, biochemical and molecular basis of TGMS will be enriched and it will be useful to manipulate the sterility / fertility by chemicals or growth regulators. This will help the breeders to get stable sterile lines for hybridization even when the environmental factors are unfavourable.

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Plates

Plate 1. TGMS lines subjected to high temperature treatment in mini phytotron



Plate 2. Induction of male sterility by salicylic acid spray (Glass House Condition)



Isozyme pattern of antioxidant enzymes in TGMS lines under fertile and sterile conditions

Plate 5. Catalase (CAT)

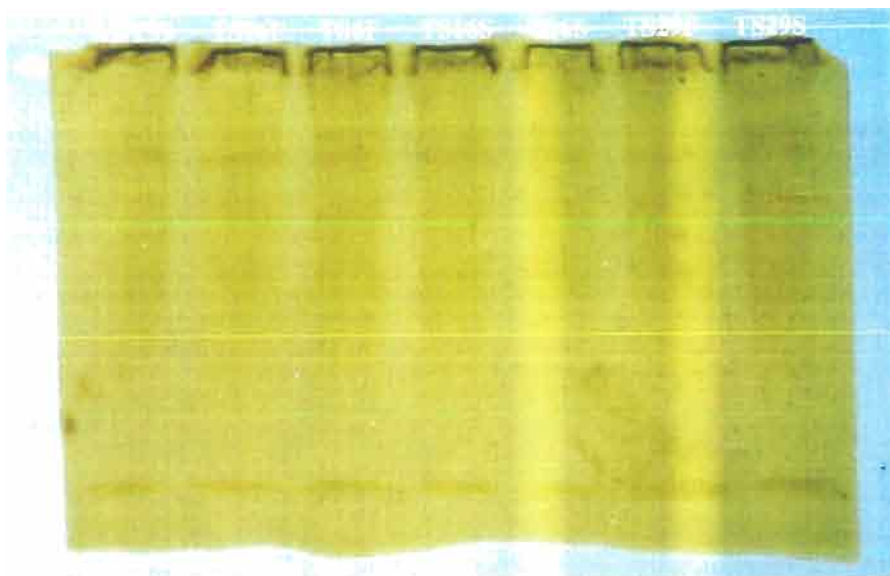


Plate 6. Peroxidase (POX)

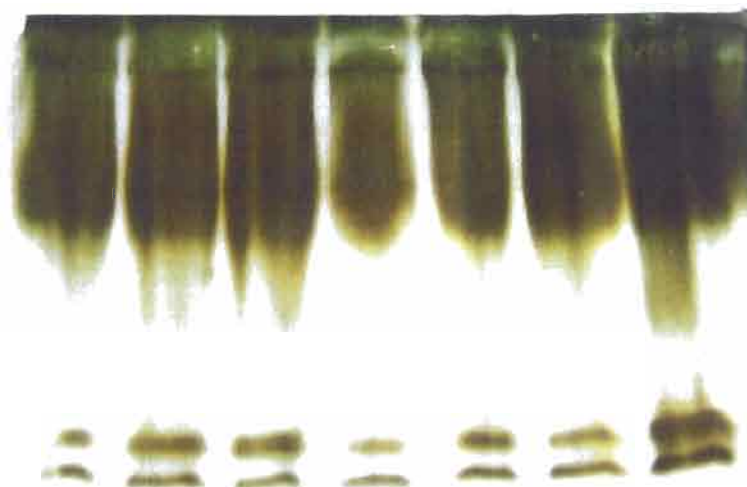


Plate 7. Superoxide dismutase (SOD)

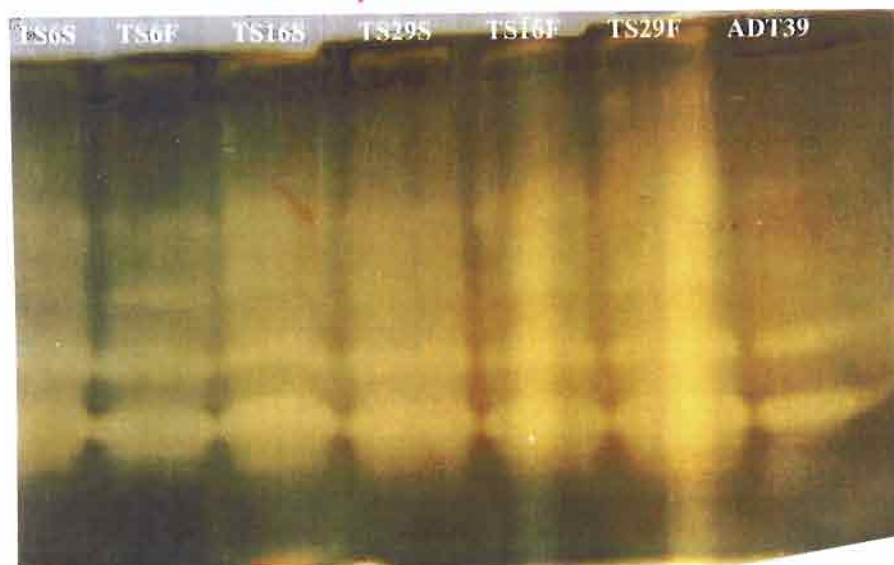


Plate 8. Isozyme pattern of Esterase (EST) enzyme in TGMS lines under fertile and sterile conditions

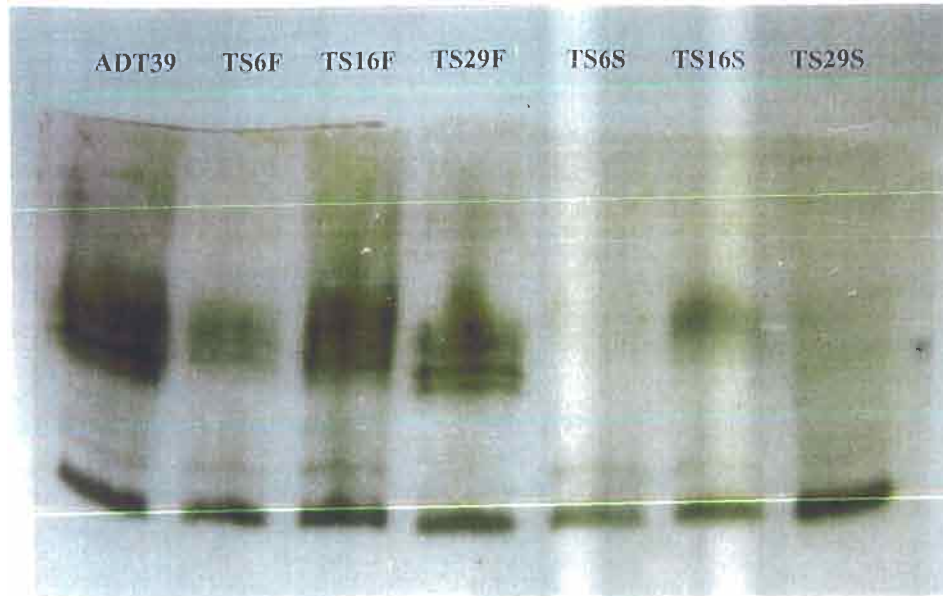


Plate 9. Isozyme pattern of Polyphenol oxidase (PPO) enzyme in TGMS lines under fertile and sterile conditions



Plate 10. Fertile and sterile anther and pollen free anthers of TGMS lines



Pollen free anthers

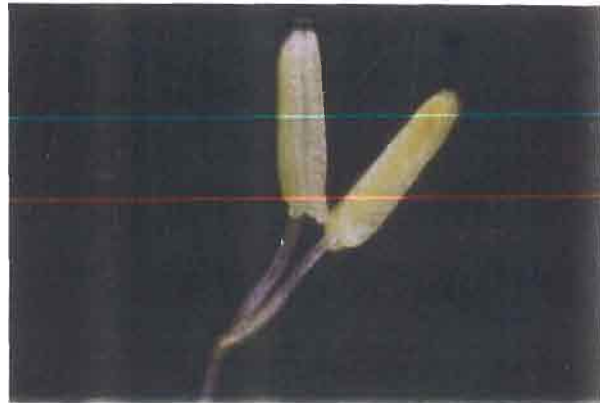


Plate 11. Fertile and sterile pollen grains of TGMS lines



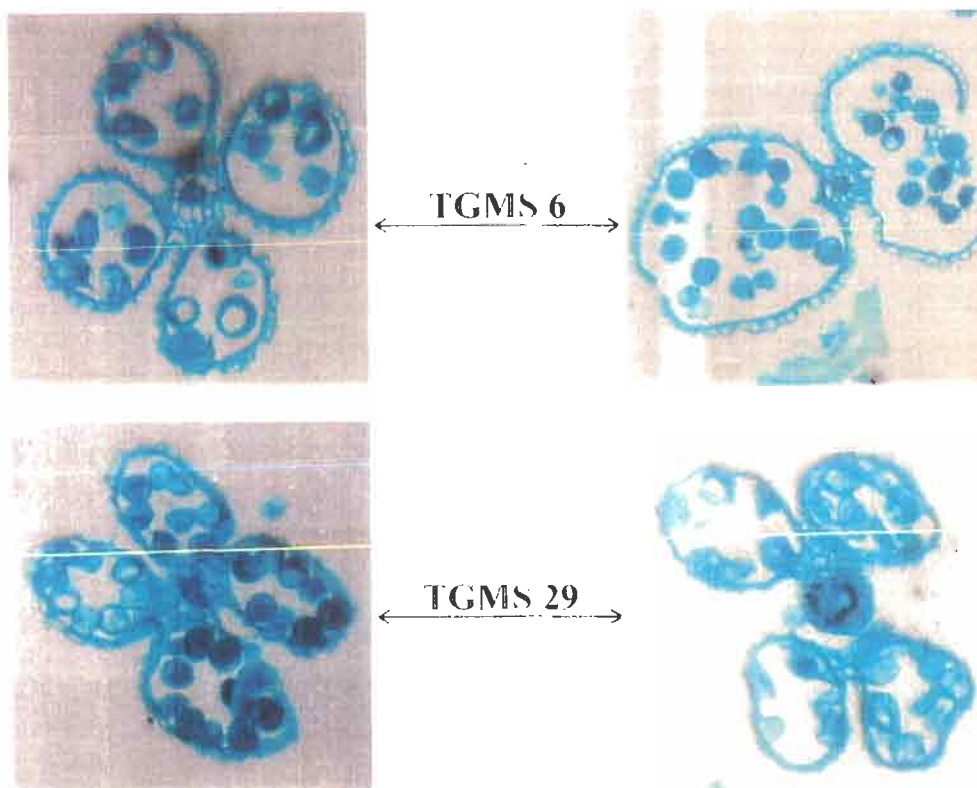
Fertile pollen grains

Sterile pollen grains



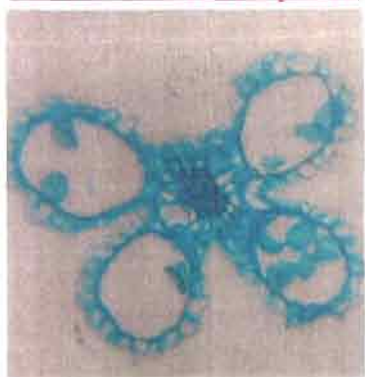
Plate 12. Microtome section of fertile and sterile anthers of TGMS lines

Fertile anthers with stained pollen grains



Sterile anther with unstained pollen grains

Anther with sterile pollen



Pollen free anther



← TGMS 6 →

← TGMS 29 →

Plate 13. Induction of male sterility by salicylic acid treatment

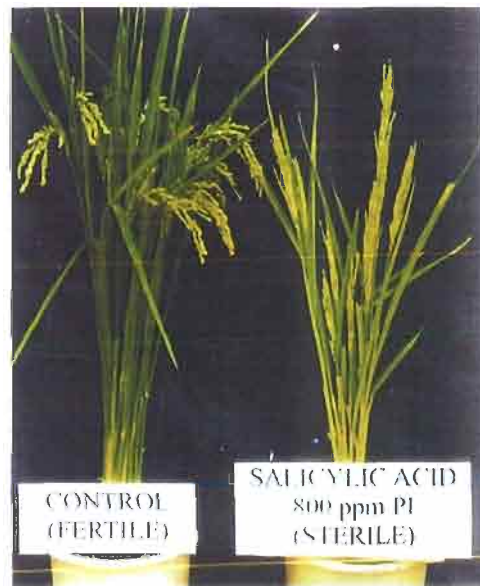
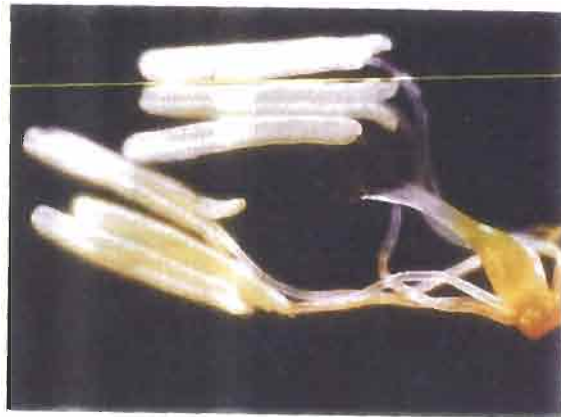


Plate 14. Salicylic acid induced male sterility in TGMS lines

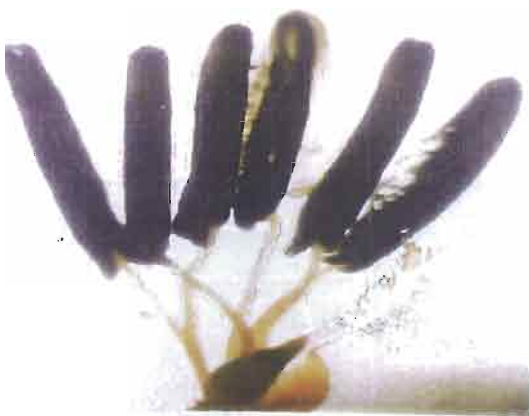
Fertile anthers with fertile ovary
TGMS 29



Sterile anthers with fertile ovary
TGMS 29



Fertile anthers with fertile ovary
TGMS 6



Sterile anthers with fertile ovary
TGMS 6



Plate 15. Salicylic Acid induced male sterile lines crossed with C-20R

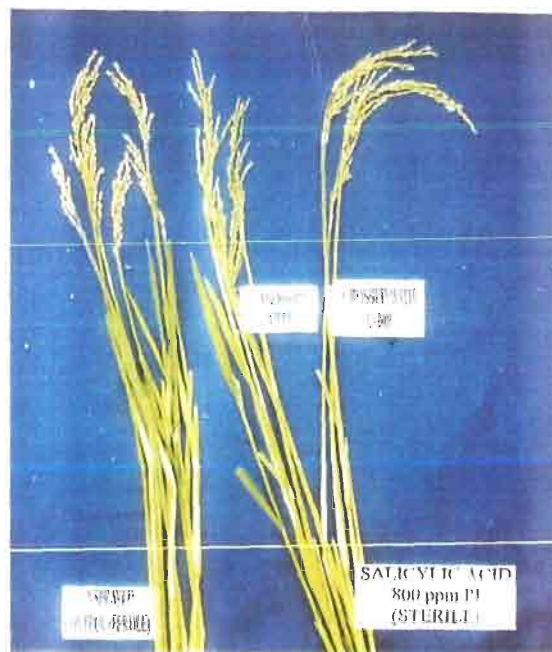


Plate 16. Induction of fertility in TGMS lines by growth regulators

