PHYSIOLOGICAL AND BIOCHEMICAL BASIS FOR STERILITY AND FERTILITY INDUCTIONS IN THERMOSENSITIVE GENIC MALE STERILE RICE LINE

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

D.VIJAYALAKSHMI, M.Sc. (Ag.)
I.D. NO : 00-808-004

DEPARTMENT OF CROP PHYSIOLOGY
AGRICULTURAL COLLEGE AND RESEARCH INSTITUTE
TAMIL NADU AGRICULTURAL UNIVERSITY
COIMBATORE – 641 003.

2003

PHYSIOLOGICAL AND BIOCHEMICAL BASIS FOR STERILITY AND FERTILITY INDUCTIONS IN THERMOSENSITIVE GENIC MALE STERILE RICE LINE

BY

D.VIJAYALAKSHMI, M.Sc. (Ag.)

DEPARTMENT OF CROP PHYSIOLOGY
AGRICULTURAL COLLEGE AND RESEARCH INSTITUTE
TAMIL NADU AGRICULTURAL UNIVERSITY
COIMBATORE – 641 003.

2003

CERTIFICATE

This is to certify that the thesis entitled "**PHYSIOLOGICAL AND BIOCHEMICAL BASIS FOR STERILITY AND FERTILITY INDUCTIONS IN THERMOSENSITIVE GENIC MALE STERILE RICE LINE**" submitted in part fulfillment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE) IN CROP PHYSIOLOGY** to the TAMIL NADU AGRICULTURAL UNIVERSITY, COIMBATORE, is a record of bonafide research work carried out by **Mrs. D. VIJAYALAKSHMI** under my supervision and guidance and that no part of this thesis has been submitted for the award of other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

Place : Coimbatore
Date :

(Dr. U. BANGARUSAMY)
CHAIRMAN

Approved by

Chairman

(**Dr. U.BANGARUSAMY**)

Members

(**Dr. G.PATHMANABHAN)**

(Dr. K.THIYAGARAJAN)

(Dr. A.MANICKAM)

EXTERNAL EXAMINER

Date :

ACKNOWLEDGEMENT

I deem it great pleasure to place on record my deep sense of gratitude and profound thanks to **Dr.U.Bangarusamy**, Professor and Head, Department of Crop Physiology and Chairman of my advisory committee for his meticulous guidance, valuable counselling and constant encouragement throughout the course of this investigation, and also in the preparation of this manuscript.

I wish to express my heartfelt gratitude to the members of my advisory committee, **Dr.K.Thiyagarajan**, Professor and Head, Department of Rice, **Dr.A.Manickam**, Professor, Centre for Plant Molecular Biology and **Dr.G.Pathmanabhan**, Professor, Department of Crop Physiology for their inspiring guidance and prudent suggestions in embelling this study.

.

I express my grateful thanks to **Dr.M.Thangaraj,** retired Professor of Crop Physiology for his keen interest and expert counselling. I extend my thanks to **Dr.S.Chandrasekar**, Professor, Department of Agricultural Entomology, TNAU for permitting me to do the HPLC analysis in his laboratory. The help extended by **Dr.V.Sashidhar**, Professor, Department of Crop Physiology, UAS, Bangalore for doing ELISA studies is gratefully acknowledged. I also thank **Dr.M.Kumar,** Assistant Professor, Centre for Plant Breeding and Genetics, TNAU for permitting me to do fluorescence microscope studies in his laboratory..

I extend my grateful thanks to all my teachers in the Department of Crop Physiology, for their valuable suggestions and cordial treatment.

I express my special thanks to my classmates **M.Djanaguiraman, Lini Alappat, M.Senthil, N.Anandaraja** and **K.Senguttuvan** for their well wishes and unsolicited assistance and valuable support rendered during my Ph.D. programme.

I am also thankful to the family members of **Dr.U.Bangarusamy**, for their cordial reception and hospitality showed during the preparation of this manuscript.

Ineffable is my fervent gratitude to my husband, **Dr.C.Karthikeyan,** my beloved son, **K.Arunachalam** and my parents for their mellifluous love and poignant affection, prayers and incessant persuasion. I am indebted to them for everything they had been to me.

I wish to express my sincere thanks to TNAU for the financial assistance rendered as SRF fellowship in the University Plan Scheme to undergo my Ph.D.(Agri) degree programme.

[D.VIJAYALAKSHMI]

ABSTRACT

PHYSIOLOGICAL AND BIOCHEMICAL BASIS FOR STERILITY AND FERTILITY INDUCTIONS IN THERMOSENSITIVE GENIC MALE STERILE RICE LINE

By

D.VIJAYALAKSHMI

Degree : **DOCTOR OF PHILOSOPHY IN CROP PHYSIOLOGY**

Chairman **: Dr.U.BANGARUSAMY**
Professor and Head
Department of Crop Physiology
Tamil Nadu Agricultural University
Coimbatore – 641 003.

Year : 2003

An investigation was carried out in a Thermosensitive Genic Male Sterile (TGMS) line, TS 29 to fix the exact sterility temperature and to identify the particular sensitive stage of rice phenophase for effective male sterility induction. The study has clearly indicated that mean temperature of 27.5^{o}C is the minimum critical sterility temperature for achieving complete pollen sterility in TS 29 and the precise thermosensitive stage for effecting complete pollen sterility is 10 to 25 days before heading.

Detailed investigations carried out to characterise the thermosensitive nature of TS 29 in terms of physiological and biochemical parameters revealed that severe reductions in IAA and ABA contents in the leaves and young panicles, and increased ethylene release rates from emerging panicles were observed in TS 29 with sterile pollens. The photosynthetic rate steeply declined,and the hill reaction rate was severely hampered in sensitive TS 29 by CST treatment.

There was significant reductions in soluble protein, amino acids, nucleic acids, ascorbic acid and nitrate reductase activity in TS 29 by CST treatment. But, starch, proline and phenols accumulated several folds. The degenerating enzymes like ascorbic acid oxidase and IAA oxidase showed higher activity causing depletion in their substrates. The reduction of free radical scavenging enzymes namely, catalase, peroxidase and superoxide dismutase showed strong association with male sterility in TS 29. The nutrients such as calcium, copper and boron were also considerably reduced due to CST treatment.

During the sensitive panicle development phase when only partial sterility occurs, salicylic acid spray at 800 ppm concentration was found to effect near complete pollen sterility in TS 29. This method is the cheapest and effective one for inducing male sterility in hybrid seed production. Salicylic acid treatment showed parallelism with CST treatment in physiological and biochemical changes leading to pollen sterility.

Spraying Brassinolide at one ppm concentration during sterile phase of TS 29 increased pollen fertility and seed set. It proved to be the most effective chemical in increasing pollen fertility and seed set percentage by favourably influencing biochemical constituents and enzyme activities.

Morphological and anatomical studies revealed shrivelled, ill-filled and malformed anthers with pollens under high temperature treatment. The distinct differences in isozyme banding patterns and protein profiles of male sterile and fertile lines of TS 29 showed potential of using them as biochemical markers for screening TS 29.

CONTENTS

CHAPTER	TITLE	PAGE
I	INTRODUCTION	
II	REVIEW OF LITERATURE	
III	MATERIALS AND METHODS	
IV	RESULTS	
V	DISCUSSION	
VI	SUMMARY	
	REFERENCES	

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE NO.
1.	Comparison of hybrid seed production systems	
2.	Pattern of fertility changes of TGMS lines in relation to temperature and day length	
3.	Fixing critical sterility temperature for TS 29	
4.	Mean temperature during different months at Coimbatore	
5.	Fixing critical stage of thermosensitivity for TS 29	
6.	Reducing sugars and total sugars in leaves of TS 29 and CO 46	
7.	Starch and total sugars in leaves of TS 29 and CO 46	
8.	Soluble protein content in leaves of TS 29 and CO 46	
9.	Proline content in leaves of TS 29 and CO 46	
10.	Total phenols in leaves of TS 29 and CO 46	
11.	Total free amino acids in leaves of TS 29 and CO 46	
12.	IAA oxidase activity in leaves of TS 29 and CO 46	
13.	Nitrate reductase activity in leaves of TS 29 and CO 46	
14.	Ascorbic acid and ascorbic acid oxidase activity in leaves of TS 29 and CO 46	
15.	Micronutrient status in leaves of TS 29 and CO 46	
16.	Effect of sterility inducing chemicals and growth regulators on pollen sterility	
17.	Effect of different phenols on percentages of pollen and spikelet sterilities in TS 29	
18.	Effect of fertility inducing chemicals and growth regulators on pollen fertility (%) in TS 29	
19.	Effect of fertility inducing chemicals and growth regulators on seed set (%) in TS 29	

LIST OF PLATES

PLATE NO.	TITLE
1.	TS 29 and CO 46 exposed to high temperature condition in Phytotron
2.	Fertile and sterile plants of TS 29
3.	HPLC profiles of phenols in TGMS 29 sterile and fertile lines
4.	HPLC profiles of polyamines in fertile and sterile leaves, and fertile and sterile panicles of TGMS 29
5.	Field trial for induction of pollen sterility
6.	Field trial for induction of pollen fertility
7.	Second field trial for studying crossed seed set using restorer line
8.	Fluorescence microscope study revealing female fertility of salicylic acid treated TS 29 line
9.	TS 29 plants showing sterile panicles with salicylic acid and fertile panicles with Brassinolide
10.	Morphological and anatomical differences between sterile and fertile anthers of TS 29
11.	SDS-PAGE of leaf and anther protein in fertile and sterile TS 29, and CO 46
12.	Isozyme pattern of esterase, peroxidase and superoxide dismutase in leaves of fertile and sterile TS 29, and CO 46

CHAPTER I

INTRODUCTION

Rice occupies a pivotal place in food and livelihood of Indian population, as it is the most important food grain in the diets of many Indians. Consumers and growers of rice contribute approximately 70 per cent of them. In India during 2000 – 2001, 134.15 million tonnes of rice was produced from an area of 44.6 million ha (FAO, 2001). By the year 2025, about 40 per cent increased global demand for rice is anticipated. Therefore, need arises to produce more rice per unit area per unit time (Virmani, 2000). To sustain this self-sufficiency and to meet the food requirements of the future, India has to increase production of 25 to 30 million tonnes of milled rice every year (Khush, 2000). Concerted national and international efforts to achieve any yield advantage in rice can considerably contribute towards food security and poverty alleviation.

Spectacular success in rice production was achieved during mid-sixties through adoption of semi-dwarf varieties, coupled with other green revolution technologies, enabling India self-sufficient in rice. Among the various possible approaches to break yield barriers in rice, hybrid rice technology is the most promising one, being practically feasible and readily exploitable as had been demonstrated in China. In the recent years, the hybrid rice growing area in China is more than 50 per cent of the total rice area of that country (Mao, 2000). The success of hybrid rice in China has encouraged scientists at IRRI and several countries around the world to explore prospects of this technology for increasing rice yields. India has earned the unique distinction of being the second nation in the world after China to make hybrid rice a commercial reality for increasing both production and productivity. Commercial hybrids in just four crops namely, maize, sorghum, rice and

sunflower have provided an additional 90 million tonnes of food production annually (Duvick, 1999). The cytoplasmic male sterility (CMS) system, also known as three-line system, is presently the most widely used technology for producing F_1 rice hybrids. Although it is effective, yet it is cumbersome, because CMS lines require specific maintainer and restorer lines. Currently, most of the commercially usable CMS lines possess wild and abortive (WA) cytoplasm induced male sterility. This situation makes CMS based rice hybrids potentially vulnerable to biological stresses. Recently, another type of male sterility, that is sensitive to the environment and is induced by the interaction of environmental factors with nuclear genes, has been identified to develop hybrids in rice in China (Yuan, 1997). This is known as environment sensitive genic male sterility (EGMS), which comprises of photosensitive genic male sterility (PGMS) and thermosensitive genic male sterility (TGMS).

EGMS plants can be used as female parents to produce hybrid seeds during the sterile phase through outcrossing, whereas, during the fertile phase, these plants are multiplied through self-fertilization without the use of a maintainer line as required in the CMS system. Since, only two lines are required for maintenance and multiplication of male sterile lines and production of hybrid seeds, it has several advantages over CMS system. Its restoring spectrum is wide and hence, the choice of parents in developing high heterotic hybrids is greatly broadened. EGMS genes can be easily transferred to any rice line with desirable characteristics (Yuan, 1996). EGMS system is capable to produce an yield of 9 to 10 t ha^{-1}, outyielding the best existing hybrids by 10 to 20 per cent.

India, being a tropical country with significant variations at different altitudes and in different seasons, TGMS system is more suitable (Ilyas Ahmed, 1996),

wherein, the genotypes become male sterile at a particular temperature and become fertile at some other temperature (Borkakati and Virmani, 1996).

Physiological characterization of TGMS lines, with respect to a range of temperature causing sterility and fertility expression, is a prerequisite for their effective and economic use to develop and commercialize hybrids. It also involves determining the 'sensitive stage' during the development and growth of TGMS lines. Though considerable information is available on the heterosis and combining ability of two line hybrids using TGMS, the physiological and biochemical basis of male sterility caused by high temperature is still to be explored. So, understanding the physiological and biochemical basis of fertility transformation of this novel source of male sterility can be an effective genetic tool in crop improvement programmes to meet the ever-increasing food demand caused by rapidly expanding populations apart from increasing incomes.

Due to greater instability of temperature than photoperiod in nature, regions where TGMS lines can be utilized in hybrid seed production are limited, because lower temperature may cause the sterility to fluctuate if its critical temperature is high. Contrarily, multiplication of the sterile lines will be affected by high temperatures if the critical temperature is low (Lu *et al*., 1994). Chemical induction of male sterility, leaving the female organs fertile, by a simple chemical treatment would be of great importance for the hybrid seed production in TGMS lines, even when the temperature fluctuates or drops below critical sterility temperature (Ilyas Ahmed, 1996). So chemical manipulation through growth regulators may help to induce male sterility more efficiently. The plants may be rendered completely male sterile with no chances for partial fertility. The concept of using chemicals to restore fertility in genetic male sterile lines has been in existence from the time there has been interest

in chemical hybridizing agents (Foster, 1981). Once an adequate male-sterile female parent seed supply is available, hybrid seeds can be adequately produced. This technology has not yet advanced to the stage, where its potential is known.

At this juncture, successful large scale hybrid rice production assumes greater significance in closing the yield gap than ever before.Since,further success and sustenance of hybrid rice in India depends primarily on TGMS system, an investigation was proposed to address the following objectives:

1. To identify the critical sterility temperature and thermosensitive phenological stage for sterility transformation of the selected TS 29 rice line
2. To study the possibility of induction of sterility/fertility in the selected TS 29 line using chemicals and growth regulators
3. To understand the physiological and biochemical basis for sterility/fertility induction by chemicals and growth regulators
4. To study the morphological and anatomical changes, associated with male sterility, induced by high temperature, and to prove the female fertility in the chemically induced TS 29 and
5. To identify a reliable biochemical marker for rapid screening of TS 29.

CHAPTER II

REVIEW OF LITERATURE

Exploitation of hybrid vigour has now become a potent tool in plant breeding. The successful utilization of this phenomenon in enhancing crop production, however, depends on the economics of hybrid seed production. At the current rate of population growth in India, the requirement of rice by the turn of the century is estimated to be around 150 m tonnes. To achieve this target, hybrid rice technology is the most feasible and readily adaptable one (Siddiq, 1996). The major limitations of the usual three line breeding approach are availability of only a few stable CMS lines with good combining ability, need for the use of maintainers and negative effects associated with sterile cytoplasm (Virmani *et al*., 1996). These limitations have prompted the scientists to develop a simple and more efficient method for the exploitation of hybrid vigour. Two line breeding is a potential alternative tool to overcome some of these limitations. Two major two-line approaches have emerged. They are (i) use of environment sensitive genic male sterility and (ii) use of chemically induced male sterility. Since there are wide temperature differences across latitudes and altitudes in India, thermosensitive genic male sterility (TGMS) is considered more feasible under tropical conditions (Lu *et al*., 1996). The chemical method for inducing sterility can obviate the often lengthy time period required to obtain male sterile and restorer lines, which usually must precede evaluation of hybrid performance (McRae, 1985).

This chapter reviews the current status of research on TGMS system for two line breeding and the physiological, biochemical and molecular mechanisms involved to explore the prospects of hybrid seed production using the most efficient chemical gametocide.

2.1. Two line breeding

Many years of practice and experience have proved that the CMS system / three line method is an effective way to develop hybrids and will continue to be important in this decade (Yuan, 1992). However, there are some constraints and problems in such a system. Scientists in China (Wu *et al*., 1991; Zhang *et al*., 1991), in Japan (Maruyama *et al*., 1991) and in IRRI (Virmani and Voc, 1991) have identified two line breeding as an alternative to the three line breeding which uses CMS system. The hybrid seed production in three line breeding and two line breeding are schematically represented in Fig.1.

Two line breeding system utilising environmental sensitive genic male sterility (EGMS) is more simple and highly efficient in hybrid rice seed production (Pandey *et al*., 1998). The lines which exhibit sterility / fertility with respect to photoperiod are called photoperiod sensitive genic male sterile (PGMS) lines and a pollen donor are needed and hence are 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 a male parent which need not possess restorer genes. The negative effects associated with the sterility inducing cytoplasm are also avoided (Ilyas Ahmed, 1996). 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).

2.2. EGMS system

EGMS lines have been classified into three categories based on their sensitivity to photoperiod or temperature by Cheng *et al*. (1996).

PGMS (Photoperiod sensitive genic male sterility)

This line is characterized by significant photoperiod (P) effect and P x T interaction and non-significant temperature (T) effect. PGMS line is sterile when the photoperiod exceeds 14 hours and the same line becomes fertile if placed under < 13 hours of photoperiod (Jauhar Ali, 1996).

TGMS (Temperature sensitive genic male sterility)

The line is characterized by significant 'T' effect, non-significant 'P' effect, with significant or non-significant P x T interaction. TGMS line is sterile when temperature is high and the same line becomes fertile when the temperature falls (Borkakati and Virmani, 1996).

PTGMS (Photothermo sensitive genic male sterility)

It is characterized by only significant P x T interaction. 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 sub type 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). Most of the PGMS lines, earlier discovered, were later reported to fall in this category (Zhang *et al*., 1992).

2.3. TGMS systems

Temperature sensitive genic male sterility (TGMS) line was identified by Chinese and Japanese scientists which was completely sterile under high temperature (> 32^{o}C), and was fertile under low temperature (< 24^{o}C) (Tan *et al*., 1990 and Maruyama *et al*., 1990a). Since the required day length difference do not exist in the

Indian sub continent, PGMS approach may not be relevant (Virmani, 1992), TGMS system has more potential for use in the tropics (Ikehashi, 1995).

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, while others were developed by irradiation breeding (Maruyama *et al*., 1991; Virmani, 1992) or were selected from filiar generations following hybridization (Zhou *et al*., 1991).

2.3.1. TGMS lines in various countries

Annong IS was the first temperature sensitive male sterile line isolated as a spontaneous mutant in Hunan province of China, which remained male sterile under high temperature and fertile under low temperature (Yuan, 1990). The lines 5460S and R59TS, which were developed in Fujian, showed fertility under 27/22^{o}C, but sterility under 33/28^{o}C (Yang and Wang, 1990). Breeders in Yunan province of China developed an inverse TGMS line IV A, which was sterile in low temperature (24^{o}C) and fertile at high temperature (27^{o}C) (Zhang *et al*., 1991). Maruyama *et al*. (1991) reported a TGMS mutant induced by 20 Kr gamma rays in Japanese rice variety Remei. The male sterile mutant designated as H89-1, exhibited no seed set under 31/24^{o}C, partial fertility under 28/21^{o}C and complete fertility under 25/15^{o}C.

The TGMS line IR 32364-20-1-3-2-B, developed from an indica rice variety at IRRI showed sterility at 32/24^{o}C but partial fertility at 27/21^{o}C and complete fertility at 24/18^{o}C in Phytotron (Virmani, 1992). Sathyanarayana *et al*. (1995) identified a mutant MSM 65-2 with 100 per cent male sterility under 30/18^{o}C and partial to complete fertility when minimum temperature was higher than 22^{o}C.

Transfer of TGMS gene from Norin PL 12 at IRRI to different genetic background resulted in different lines with TGMS character namely, IR 68945, IR 68949, IR 70118 and IR 68294 (Siddiq, 1996), IR 68946-1-13-15, IR 71018-13-13 and IR 68948-4-14-1-4 (Lu *et al*., 1996). The different TGMS lines developed in China by Lu *et al*.(1996) were GDS 2S, KS IS, 6445, Shuguang 61S, W 916075, Anxlang S, Xlang 15S and 9201, from an indica rice variety NK 58S.

Anxiang S and Xiang 125S from cross derivative – Annong IS, and 9201 from cross derivative – 5460S were TGMS lines developed by Lu *et al*. (1998). GD 2S, KS 15, 6442 S, Shuguang 612S, W 91607S were TGMS lines developed by Lu *et al*. (1998) from cross derivative – Nongken 58S. A new TGMS line Yi-DIS was developed by Lu *et al*. (2000).

2.3.2. TGMS lines developed in India

Rangaswamy (1992, 1994) first reported six TGMS lines in 1992 and five more lines in 1994 from Coimbatore. Ali *et al*. (1995) isolated six TGMS lines namely, SM 3, SM 5, F 61, JP 2, JP 18-1A-12 and SA2 (A3), which remained sterile at high temperature and turned fertile at low temperature. Later, Ali *et al*. (1996) reported that J 24A (Pusa 33-16 A) also showed TGMS character. Different TGMS lines like IRTG 5, IRTG 6, TGMS 1, TGMS 2 and ATG series were identified from Directorate of Rice Research (DRR), Hyderabad (Anon., 1995).

Reddy *et al*. (1996) reported a new TGMS line IC 10 from Norin PL 12/IR 36. Saxena and Singh (1996) reported the TGMS nature of IR 68292 and IR 68945 from Pantnagar. In Coimbatore, Elsy and Rangaswamy (1996) evaluated promising TGMS lines and reported that TGMS 6, 9, 10, 16 and 18 showed sterility / fertility variations according to changes in temperature. Thiyagarajan *et al*. (1996) identified TGMS 15

and TGMS 16 as stable and promising lines for hybrid seed production based on the study conducted simultaneously at Coimbatore and Gudalur. Vijayakumar *et al*. (1996) reported the promising TGMS nature of IR 68945-4-33-14, IR 68948-12-3-7 and IR 68949-11-5-31 from DRR, Hyderabad. Rangaswamy and Jayamani (1997) identified five TGMS lines namely, TGMS 16, a selection from IR 68945, and TGMS 32, TGMS 33, TGMS 35 and TGMS 36 were selections from C 41, C 42, C 44 and C 45.

Malik *et al*. (1997) reported three traditional rice lines with TGMS nature which showed complete pollen sterility at daily mean temperature of 27.5^{o}C (32/23^{o}C). Saxena and Singh (1998) reported three promising sources of TGMS namely, IR 68292-10-34-25, IR 68949-5-31-34 and IR 68945-33-4-14-7, which showed complete sterility when sown during February to May and partial fertility when sown during June and July. Rongbai and Pandey (1999) identified UPRI 95-140 which exhibited complete pollen sterility at daily mean temperature above 27.1^{o}C. Ali *et al*. (2000) reported two new TGMS lines, JP 24A and JP 24B from WA based Pusa 33-16A line. These lines showed typical sterility point at 33.8^{o}C and 35.1^{o}C respectively. Various TGMS lines reported in India are summarised in Table 1.

2.4. Factors influencing fertility transformation of TGMS lines

The external and internal factors causing the changes of fertility of TGMS rice is complex and has attracted the attention and interest of the scientists all over the world working on hybrid rice seed production. 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. The fertility of TGMS lines reacts

differently to light and temperature when identical sterile lines were situated in different ecological environments (Yuan, 1990).

2.4.1. External factors

The transition of pollen fertility in TGMS lines is mainly induced by temperature. When temperature is between critical value of sterility and fertility, long day length may induce sterility and short day length induce fertility (Yuan, 1990). The higher temperature at which maximum fertility is achieved is called critical sterility temperature (CST), and the temperature at which maximum fertility is achieved is called critical fertility temperature (CFT) (Yuan, 1990; Ali, 1996). The CFT 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 CST 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 multiply during a specific season or at higher altitudes in tropical rice growing areas (Zhang *et al*., 1993).

The pattern of fertility changes of TGMS lines in relation to temperature and day length is shown in Fig. 2.

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 for different agroclimatic regions (Yuan, 1992). In TGMS lines, the critical high temperature inducing sterility was 25–27^{o}C (Yuan, 1990). At temperature of 25^{o}C, pollen fertility of all plants was resorted to normal regardless of day length (Cai *et al*., 1991). Xue and Shen (1991) reported that a decrease of 4^{o}C in temperature during light period increased pollen fertility, but a change during dark had no effect. They found no significant difference between lines in pollen sterility, but temperature x line showed significant difference.

Chen *et al*. (1993) reported that night temperature was more important in the expression of thermosensitivity than day temperature. The TGMS lines were sterile when maintained at 31/28^{o}C and fertile at 24/22 ^{o}C. Reddy *et al*. (1996) reported that TGMS lines reverted back to fertility under cloudy weather and cooler night temperature. Thiyagarajan *et al*. (1996) evaluated TGMS 15 and TGMS 16 in Tamil Nadu simultaneously at Coimbatore (high temperature zone 38/23 ^{o}C) and Gudalur (low temperature zone 30/16 ^{o}C) during summer, 1995. The lines showed 100 per cent pollen sterility at Coimbatore, whereas 55 – 80 per cent pollen fertility was observed at Gudalur. CFT for indica TGMS lines ranged between 24-28^{o}C. Comparative effects of treatments revealed that the maximum temperature in day/night combination was crucial in deciding the sterility or fertility of TGMS lines. The critical temperature for fertility alteration in different TGMS lines is given in the Table 2.

2.4.1.1. Classification of TGMS lines based on critical temperature

Commercial exploitation of TGMS lines in two line breeding requires the understanding of critical temperature at which a given line will be male sterile or male fertile for selecting the season for its multiplication or hybrid seed production. Based on critical sterility temperature (CST) and critical fertility temperature (CFT), TGMS lines have been classified mainly into four types (Ali *et al*., 1995).

Type 1 : High CST (> 32^{o}C) low CFT (< 24^{o}C): Such types were reported to be safe for both hybrid seed production and maintenance, eg. lines SM3 and SM5.

Type 2 : High CST (> 32^{o}C) high CFT (> 24^{o}C): Such lines could be useful under tropical condition only. eg. JP-2 and W7415 S.

Type 3 : Low CST (< 32^{o}C) low CFT (< 24^{o}C): Such lines are ideal for tropical situation and could be used in hybrid seed production without any

problem. CST ranged from 30-32^{o}C and CFT from 20–24^{o}C, eg. JP 8-1A-12, SA 2 and F 16.

Type 4 : Low CST (< 32^{o}C) high CFT (> 24^{o}C): No specific lines identified in this category.

2.4.2. Critical stage of TGMS lines

The thermosensitive period of fertility alteration of TGMS lines are important in selecting the sowing time for hybrid seed production or seed multiplication, so that the critical temperature coincides the critical thermosensitive stage. Yang *et al*. (1995) studied the relationship between air temperature and percentage of sterile pollen in seven indica PTGMS lines and found a critical thermosensitive stage occurring about 20 days before heading and another at meiosis stage. In TGMS lines, the temperature during the meiosis stage of PMC was critical for fertility or sterility in plants (Chen *et al*., 1992). Whereas, Chen *et al*. (1993) reported the stage most sensitive to temperature was gametogenesis to formation of PMC to the early monokaryon pollen stage. Thus, the critical stages of fertility alteration in different PGMS / TGMS lines are given in Table 3.

2.4.3. Genetic basis of TGMS

The genetic basis of environment sensitive genic male sterility (EGMS) has been widely studied and reported. All the results indicated that the nuclear genes controlled the sterility of EGMS and there is no cytoplasmic effect on it. EGMS is reportedly governed by a single recessive gene in most crops except in wheat and vaccinium, in which, it was controlled by three recessive genes or by polygenes (Kaul, 1988). In rice, photosensitive genic male sterility is controlled by a single recessive gene (Shi, 1985; Feng *et al*., 1985; Shi and Deng, 1986). Two pairs of

recessive genes controlling PGMS was reported by some workers (Shen, 1992; Yang and Chu, 1995; Yang, 1997; Wan and Ma, 1997). Xue and Deng (1991) reported quantitative inheritance of PGMS in their studies. Two pairs of recessive genes influenced by many minor genes in the control of PGMS have been reported by Lu *et al*. (2000).

TGMS has been reported to be controlled by a single recessive gene in most of the lines. Sun *et al*. (1989) reported that the TGMS in 5460 S was controlled by a single recessive gene tms 1. Subsequently, Maruyama *et al*. (1991) studied the inheritance pattern of an irradiation – induced mutant Norin PL 12. Borkakati and Virmani (1993) found that the TGMS trait of IR 32364 mutant was controlled by a single recessive gene. Borkakati and Virmani (1996) also studied the segregation behaviour for pollen and spikelet fertility of F_1, F_2 test cross and F_3 generation of crosses of Norin PL 12 and IR 32364. They have indicated that the TGMS trait in two mutants was controlled by a single recessive gene.

Double recessive genic control of TGMS was reported in TS 15, TS 16 and TS 18 by Sampoornam and Thiyagarajan (1998) and Cherian (1998). Rongbai and Pandey (1999) attempted to analyse the genetics of TGMS in the line UPRI 95 – 140 TGMS and its breeding behaviour in diverse genetic backgrounds of male parents namely, IR 36, UPRI 95-117 and UPRI 95-161. They inferred that two pairs of recessive genes in duplicate fashion controlled the inheritance of TGMS trait causing diverse recombinant TGMS plants in the segregating population with very low selection frequency. Contrary to the above report, F_2 of this line with 44 genotypes of normal male fertility indicated monogenic, digenic and trigenic inheritance with frequencies of 18 per cent, 52 per cent and 29 per cent respectively (Rongbai and Pandey, 2000).

Ali *et al*. (2000) made crosses among TGMS lines (ID 24 x SM 5 and IC 10 x JP 4) and found that the F_1s were completely normal for pollen and spikelet fertility under high temperature conditions. The F_2 segregation showed 9 fertile : 7 sterile ratio, thus indicating the character to be governed by two separate genes. Viraktamath *et al*. (2000) studied the genetics of ID 24 in the F_2 generation of 28 crosses made using popular high yielding varieties of indica. The results showed that a single recessive gene, sensitive to temperature, affected the male sterility / male fertility in this line. Single recessive gene controlling male sterility has also been reported in the TGMS line F 61 and SA 2 by Hussain *et al*. (2000). Wu (2000) has found two genes responsible for decreasing the temperature at which male fertility is induced in TGMS genotypes. These markedly reduced the level of contamination of hybrid seeds with selfed seeds.

2.4.4. Internal factors affecting TGMS system

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 (Bowler and Chua, 1994). 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 and biochemistry 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.

2.4.5. Physiological changes affecting TGMS system

2.4.5.1. Hormonal changes

Zhao *et al*. (1996) reported that indole acetic acid (IAA) deficiency in the reproductive organs at early developmental stages and reduction of GAs in later developmental stages were the factors causing anther sterility in Nongken 58 S 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^{o}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 anther than in fertile anthers of parents Nanjing I and Qui gang respectively (Zhu and Cao, 1997).

Hasegawa *et al*. (1995) have quantified the endogenous gibberellins in anthers of normal and male sterile lines of Nihonmasari by immuno assay. The content of GA 4/7 increased rapidly, 1 – 2 days before anthesis. The content of the GA_3 in male sterile lines was roughly correlated with the pollen developmental stage characteristic of each male sterile line. It was concluded that gibberellins are related to pollen and anther development.

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

than in fertile condition in young panicles. Li De Hong *et al*. (1996) reported that the ERR was negatively correlated with pollen fertility and was low in long day low temperature (25 ^{o}C) and high under short day high temperature (32 ^{o}C). Tian Chang En (2000) studied the ethylene release rates in leaves and panicles and reported that the excess release of ethylene might be a cause of CMS in rice.

Changes in endogenous levels of phytohormones under stress condition have been considered as a step towards adaptation to the adverse conditions (Abbas *et al*., 1999). Synthesis of ABA, in general, is upregulated with the onset of high temperature stress (Wright and Hiron, 1969). In the rice panicles, superior spikelets display higher growth rates than the inferior spikelets during the initial phase because, ABA content in the superior spikelets increased rapidly after flowering, while in the inferior spikelets ABA content increased slowly. Thus Tsukaguchi *et al*. (1999) has shown that ABA is involved in assimilate partitioning among spikelets in a panicle during the initial phase of grain filling of rice.

2.4.5.2. Photosynthetic rate

The selection of big panicles and high photosynthesis function of leaves are important for breeding purpose. Chen Jianmin *et al*. (2001) revealed that exposure of isolated thylakoids or intact plants to elevated temperature was known to inhibit photosynthesis at multiple sites. Chlorophyll a fluorescence transient analysis in intact rice leaves indicated a loss in PS II photochemistry and an associated loss in a number of functional PS II units (Vani *et al*., 2001). Synthesis of RuBP carboxylase and several thylakoid proteins responsible for photosynthetic electron transport and photophosphorylation was greatly inhibited at high temperatures (Maruyama *et al*., 1990b).

Tong *et al*. (1992) studied the changes in photosynthetic activity of NK 58 S and found that long day treatment decreased photochemical activity and efficiency of light energy transformation in PS II 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 58 S plants. Sterility inducing condition decreased photochemical activity and efficiency of light energy transformation in PS II and markedly reduced the electron transmission of chloroplasts. Chloroplasts from Nongken 58 S 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 58 S 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 were 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 stage 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 PS II 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 if rice was exposed to both high temperature and increased CO_2 concentrations. Ma Dehua *et al*. (1999) studied the effect of high temperature stress on cucumber seedlings and reported that the chlorophyll a, net photosynthetic rate and stomatal conductance were markedly reduced.

2.4.5.3. 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 micronutrient deficiency, exhibit reduction in anther size and pollen abortion. The pollen grains are poorly developed, small, shrivelled, highly vacuolated and lack proper exine and cytoplasmic contents. In bean cultivars, the contents of Mg and Ca increased a little, while Na, K, Mn, Fe and Zn reduced under high temperature (Moreno-Limon *et al*., 2000).

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 contents of soluble calcium in the leaves and young panicles of Nongken 58 S were 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 surfaces of pollen grains.

These anomalies in the distribution of calcium accumulation correlated with the failure of pollen development and pollen abortion (Tian *et al*., 1998).

2.4.6. Biochemical changes affecting TGMS system

2.4.6.1. Amino acids and proteins

Amino acids and other soluble nitrogenous compounds play essential roles in plant metabolism, and get altered under temperature stress (Stewart and Larher, 1980). Characteristic differences in bound and free amino acids have been detected between male sterile and male fertile plants. Kern and Atkins (1972) have reported a reduction in the amounts of leucine, isoleucine, phenyl alanine and valine in male sterile plants of barley and rice, while Rai and Shoskopf (1974) have reported an increase in asparagine and glutamic acid in wheat male sterile lines. Alanine, glycine, arginine and aspartic acid were increased in male sterile lines of wheat, rice, sorghum and cotton respectively (Kaul, 1988).

Proline accumulation occurred in all cultivars under all stresses and increased with increasing duration of stress. Sensitive cultivars accumulated more proline than tolerant ones (Liu *et al*., 2000). Xiao *et al*. (1993) reported that the contents of free proline in the fertile anthers were higher than the sterile anthers and abnormal metabolism of free proline in 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, 16 and 18. Roystephen and Thangaraj (2000) reported the same results in TGMS 6 and 16, with a reduction of free amino acid content in TGMS lines.

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

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 58 S at young panicle development stage by D, L^{-14} C leucine labelling and two dimensional electrophoresis. The plants were treated for 10 h at 34.6^{o}C or 14 h at 33^{o}C with 28^{o}C at night. About 200 protein bands with isoelectric focusing 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 photoperiod (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 of panicle differentiation, the contents of protein amino acids in the leaves of Nongken 58 S decreased slightly, wherein, each amino acid and total free amino acid 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 64 S during high temperature periods. Huang *et al*. (1996) also reported that the soluble protein content of anthers at 30^{o}C was reduced in TGMS lines Erjiuquing S and Annang S and all pollen grains. SDS – PAGE showed bands with 13, 13.5, 23.5 kDa being absent in Erjiuquing S compared to its source line. At 30^{o}C, 12 kDa band was absent compared to 25^{o}C. Liu and Xue (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 of molecular weights of proteins 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 GDIS and N 19S were investigated under long day high temperature, short day low temperature regions, and compared with normal variety Genghao 89. Only in 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 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.

2.4.6.2. Nucleic acids

The specific changes in protein and enzymes would have resulted due to impaired transcription and post transcriptional modifications. Many scientists have tried to study the change in ribonucleic acids, where the alteration would be mainly in mRNA level under sterility. In PGMS lines, the amount of total RNA from long photoperiod treatment (14 hours) was 6.3 to 100 per cent lower than that from SD treatments (Yang and Zhu, 1990). They observed a small molecular weight RNA fraction in LD at fertility transformation. Lu *et al*. (1993) studied the changes of nucleic acid in leaves of Nongken 58 S and Nongken 58, treated with LD and SD. It was showed that the RNA content from the fifth leaf to seventh leaf of these lines both under LD and SD inductions declined gradually and equally, while RNA content in leaves above the seventh leaf segment increased differentially.

The total RNA and DNA contents of TGMS indica rice of sterile lines were much lower than that of fertile ones and the normal variety Zike at PMC and PMC meiosis stages (Shu and Chen, 1999). Roystephen and Thangaraj (2000) studied the RNA contents of fertile and sterile lines of 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. Senthil (2001) studied the DNA and RNA contents of leaves and panicles of TGMS 6, TGMS 16 and TGMS 29 under fertile and sterile conditions at different stages and reported that the DNA and RNA contents of all the lines were high under fertile condition compared to sterile condition.

2.4.6.3. Carbohydrate fractions

The starch to sugar interconversions and the sugar transport are some of the basic factors deciding the development of panicle. Peng and Wang (1991) reported a

reduction in soluble sugar content of anthers and spikelets in Nongken 58 S 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 58 S. Wang *et al*. (1991a) studied the behaviour of Nongken 58 and Nongken 58 S under 10 hours to 14 hours day length during photosensitive stage. In normal line, 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. 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. Thus, it was suggested that lack of transport of carbohydrates of the stamen might account for the male sterility. He *et al*. (1993) also reported that the content of reduced sugar at every developmental stage of young panicle in the leaves under sterility (LD) was more than that under fertility (SD) in Nongken 58 S.

Roystephen and Thangaraj (2000) reported that the carbohydrate fractions, such as, reducing sugars, total carbohydrates and total sugars were constantly increased from 25 Days Before Heading (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) and Senthil (2001) in TGMS lines. Kobayashi and Saka (2000) have also reported that the carbohydrate content in the leaf blades was closely correlated with the rate of ethylene evolution in the young rice seedling, which in turn is a measure of male sterility.

2.4.6.4. Enzymes

Plants contain a complete armoury of enzymes. Shen and Gao (1992) studied the alterations of some enzyme activities in PGMS line Nongken 58 S and reported that the activity of Nitrate reductase was decreased under long day length with increased temperature condition and the activity of superoxide dismutase (SOD) also decreased during sterile condition compared to fertile condition. During the development of anthers, the peroxidase (POX) 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 and superoxide dismutase were closely related with the transformation of fertility in Nongken 58 S (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.

Zhang *et al*. (1994a) reported that the activities of POX, catalase (CAT) and SOD were higher in fertile phase than in panicles and anthers of PTGMS rice from sterility to fertility transformation. Ascorbate oxidase, glutathione reductase and glucose-6 phosphate dehydrogenase activities increased in the fertile anthers with maximum activities 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, 9 and 22 per cent activity respectively in male sterile anthers. There was a decrease in malic enzyme, malate dehydrogenase (MDH) and isocitrate dehydrogenase activities 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 peroxidase activity in fertile and sterile anthers of indica and japonica rice hybrids and reported that the peroxidase 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 decreased during sterile condition compared to fertile condition in TGMS line 'Pei ai 64S'. Chen and Zhou (1997) studied the activities of certain enzymes in leaves of photo and thermosensitive genic male sterile lines and revealed that the activities of NRase and cytochrome oxidase increased, and acid phosphatase, peroxidase activities 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 at 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. Roystephen (1998) reported that the activities of enzymes IAA oxidase and ascorbic acid oxidase were higher under sterile condition in TGMS lines. Roystephen and Thangaraj (2000) reported that the free radical scavenging enzymes namely, super oxide dismutase, peroxidase and catalase showed reduced activities in sterile TGMS lines. Similar results were also reported by Senthil (2001).

2.4.6.5. Polyamines

Role of polyamines on fertility transformation was studied by determining endogenous polyamines (PAs) and applying PAs biosynthetic inhibitors to plants of HPGMR line Nongken 58 S (Mo and Li, 1992). The plants under red + far red + red light had a pollen stainability of 60.9 per cent and selfing fertility of 18 per cent which were 59.2 per cent and 7.5 per cent in red light and 42 and 12.9 per cent in red + far red light. Photosensitive pigments had different regulatory effects on the PAs at various developmental stages. The regulatory effects of PAs on fertility

transformation were related to the ratios of PAs of which the content of spermidine to spermine and spermine to spermidine ratio were significant. Application of PAs biosynthetic inhibitor could reverse the effect of red light on fertility transformation. During the development of young panicles, the contents of polyamines in panicles increased upto differentiation of secondary rachis branch and then reduced. Putrescine content increased in sterility and spermine content under fertile conditions, in C 407S rice (Feng and Cao, 1993). Marcel Aribaud and Tanguy (1994) have reported that in fertile plants of chrysanthemum, spermidine conjugates were predominant during floral initiation, whereas in male-sterile plants, only putrescine conjugates were detected. In both cases, ornithine decarboxylase (ODC) was involved in regulating floral initiation in normal and male sterile plants. According to Zhu *et al*. (1999), raising the temperature at 6 – 7 weeks after full bloom in apples reduced the contents of spermidine and putrescine, but did not influence spermine. Conversely reducing the temperature increased the spermidine and putrescine levels, and produced a decline in spermine.

Plants are constantly challenged by unfavourable fluctuations in their environment and the accumulation of low molecular weight metabolites such as polyamines is a universal response to cope with various forms of environmental stresses such as extreme temperatures (Vernon *et al*., 1993).

2.4.6.6. Isozymes

Rice is not only one of the important food crops, but also a model plant for study of molecular developmental biology. Isozymes have a noteworthy impact on regulating plant growth and development (Li *et al*., 1994). Correlations between differences in isozyme patterns and male fertility under different natural conditions, day length and temperature regimes were studied by means of pollen fertility index

using PTGMS line Nongken 58 S, Shuang 8-S and N 98S by Hu and Wan (1991). Linkage relationship between isozyme gene and male sterility genes were also studied. They observed a close relationship between Adh-1, Est-3 and male sterility. One of the sterility genes was linked to 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 band of four enzymes analysed, but decreased their activity (Tong *et al*., 1993). But, Liu *et al*. (1993a) observed differences in banding patterns of peroxidase isozyme in Nongken 58 S in SD and LD at stages I and IV. At stage II, LD treated plants gave two extra weak bands compared to SD treatment, whereas 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 58 S 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 105 S and 105 as in the case of Nongken 58 S and Nongken 58 was studied by Wu and Xiao (1993).

Li *et al*. (1994) analysed vertical PAGE at different plant growth stages to determine specific proteins of isozymes in TGMS rice, Annong IS. They observed a specific esterase band (S band) in Annong IS and N42 S (a PGMS line from Nongken 58 S). Annong I and 210, other normal varieties did not have the 'S' band. They reported stable expression in mature leaves and unstable expression in young leaf blades. But Est-1 and Est-2 isozyme bands were detected at all growth stages of Annong IS and Est-3 band only at mature leaf stage by Li *et al*. (1995). Li Peng *et al*.

(1997) studied the banding pattern of acid phosphatase and MDH isozymes in TGMS line Pei ai 64 S, 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 16 and 18 (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. Senthil (2001) reported appreciable discrimination in banding pattern between fertile and sterile genotypes, and may be taken as biochemical marker for identifying sterile and fertile genotypes.

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

China is probably the only country where gametocides are being used in commercial hybrid seed production. 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. 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 (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 effects with no residual toxicity which would be harmful to human beings and animals; economically easy to apply (Ali, 1993, 1996).

The following four classes of chemical hybridizing agents (CHA's) known are:

1. Plant growth regulators and substances, which disrupt floral development. eg. plant hormones, such as auxin, gibberellins and ABA. 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, phenyl cinnoline carboxylates and genesis R.
4. Inhibitors of pollen fertility, such as, acetidine-3-carboxylate (Cross and Schulz, 1997).

More than 50 chemical emasculators have been tested on more than 40 crops. But, most of them damaged the pistil or caused abnormal flowering (Wang *et al*., 1981; Yu *et al*., 1991). In the 1970s and 1980s, China developed chemical emasculators using arsenates, such as male gametocide 2 (sodium methyl arsenate). While they caused excellent emasculation of rice, they were also very toxic to the environment.

Aswathanarayana and Mahadevappa (1991) reported that 800 ppm of GA, 8000 ppm of ethephon, 0.02 per cent maleic hydrazide and 0.8 per cent 2,4-D induced a high level of male sterility in rice. Kitaoka *et al*. (1991) reported that 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 2500 ppm. The pyridine derivative hybridizing agent TO_3, applied at 0.03 mg 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 at 5 – 11 days before panicle emergence. The main effect of TO_3 was

reduction in number of stamens and they became small producing few pollen grains in rice (Zhang *et al*., 1995). Treatment at the meiotic stage was more effective for inducing male sterility. 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 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 per cent) for inducing male sterility on rice plants cv. AKS 1 at tillering and initial booting stage. The lowest concentration (3 per cent) of the detergent induced the highest level of male sterility (93.3 per cent) at tillering. At initial booting stage, highest level of male sterility was noted for the highest detergent concentration (6 per cent). Jayachandran *et al*. (2001) reported that five per cent plant extract of *Lantana camara* resulted in high spikelet sterility in rice.

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 (Mc Murray 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).

Many scientists have reported that ethephon induced male sterility of crops (Bennet and Hughes, 1972; Perez *et al*., 1973; Hughes, 1976; Parmar *et al*., 1979; Chan and Cheah, 1983). Ladislav Dotacil and Marie Apltauerova(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. Thakur and Rao (1988) reported that foliar spray of 2000 ppm ethrel on pearl millet at late boot stage increased the male sterility than at early boot stage without affecting the female fertility. Tian *et al*. (1999) reported reduction in pollen fertility by application of ethrel or ACC in CMS rice and it could 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 by inducing the highest pollen sterility with least phytotoxicity. Tian Chang En (2000) revealed 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 while partially restoring the pollen fertility in the CMS line.

In experiments with cucurbits, Wittwer and Hillyer (1954) could suppress the male flowers with maleic hydrazide, while the female flowers seemed normal and fertile. Rehm (1962) stated that 25 – 50 ppm triiodobenzoic acid induced male sterility in tomato for eight weeks without damaging the female fertility, while maleic hydrazide 500 ppm acted on the pollen of the same crop and caused male sterility for two weeks. Raman and Natarajan (1999) reported that spraying of maleic hydrazide @ 0.8 per cent on egg plant first on 20th day after sowing, second when the flower buds appeared and third spray at ten days thereafter brought about 40 -–70 per cent of pollen sterility. Manjula and Ibrahim (1999) tested the effect of ethrel, maleic hydrazide (MH) and streptomycin on pollen sterility in rice and the results revealed that MH only induced high pollen sterility and caused anther deformation and thus

high spikelet sterility. Spraying of 100 ppm maleic hydrazide resulted in 50 per cent pollen sterility in chillies (Salgare, 1995).

Kho and De Bruyn (1962) found that dichloro acetic acid solution (0.5 per cent) applied before two weeks of flowering had a positive action on the pollen sterility of Anthurium, when applied two weeks before flowering. 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). In winter rye, 2,3-dichloro isobutyrate application has been shown to affect the development of pollen (Aung, 1977). Two chemosterilants, RH 531 and RH 532 induced complete sterility in barley and wheat when applied as a foliar spray (Rajendra and Bates, 1981). 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 pigeonpea. 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 positive effect of EMS on barley in inducing male sterility, and recommended the same for large scale hybrid seed production in highly self pollinated crops.

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, GA_3 and salicylic acid was conducted either one day before or one day after pollination. The growth regulators 2,4-D and GA_3 had favourable effects on pollen tube growth, whereas, salicylic acid, an immuno suppressant, had inhibitory effect (Manickam and Sarkar, 1999). Earlier, Seetharaman and Kusumakumari (1975) reported a high degree of male sterility in

sunflower with the application of GA_3 at 90 – 100 ppm on the 3rd day after flower bud initiation. Eenink (1977) obtained male sterile flowers in all GA_3 treated lettuce plants regardless of GA_3 concentration and frequency of spraying. Some of the male sterile flowers had florets with shrivelled anthers, which released no pollen while others had no anthers at all. All florets seemed to have normal pistils. 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 lines. Senthil (2001) also reported that two sprays of salicylic acid @ 800 ppm one during panicle initiation and the second spray ten days thereafter brought about 100 per cent sterility in TGMS lines.

2.6. Induction of fertility in TGMS lines using chemicals and plant growth regulators

The concept of using chemicals to restore fertility in genetic male sterile lines has been in existence from the time there has been interest in chemical hybridizing agents (CHA). Much attention has been drawn 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 fertility are atleast in three ways. Firstly, the chemical need not be 100 per cent effective, since its use is only to increase supplies of female parent seeds. The more effective it is, the more efficiently seed could be multiplied. Secondly, all the seeds produced are exactly true to type and thirdly, since the chemical is not used in plants meant for hybrid seed production but only in plants meant for multiplication, a lower quantity is required compared with the quantity of CHA required (Laible, 1974). Once an adequate male-sterile female parent seed is available, hybrid seed is produced (McRae, 1985).

Raza and Gouri (2000) reported that the foliar application of 0.5 per cent IAA and 5 per cent ascorbic acid to rice cv. Thulasi brought about higher seed set. Sanaa *et al*. (2001) obtained more number of seeds in *Phaseolus vulgaris* plants treated with 0.1 and 0.2 mM NAA and 2 and 5 mM salicylic acid. Geetha *et al*. (2000) found that foliar sprays of GA and NAA at 100, 200 and 300 ppm and IAA at 25, 50 and 100 ppm increased the seed yield and seed weight per plant in China aster. Spraying rice with ABA on the second day after anthesis increased the grain filling rate (Yang *et al*., 1999). Wang Ding Ping (1999) reported that application of natural brassin to male-sterile rice line Xieqing zao increased the seed production by 10 per cent compared to the control.

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

The productivity of rice depends on various physiological and biochemical events that occur at different growth stages. Regulation of these parameters is one of the ways to increase the productivity.

Janda *et al*. (1998) reported that spraying of young maize plants with 0.5 mM salicylic acid showed significant decrease in net photosynthesis, stomatal conductance, transpiration and intercellular CO_2 concentration at normal growth temperature. On the contrary, higher contents of Rubisco and photosynthetic rates with high chlorophyll and hill reaction were observed in the rice plants treated with salicylic acid (Maibangsa *et al*., 2000). In *Panicum milvaceum*, an increase in net photosynthetic rate with an increase in salicylic acid was observed by Radha Bisht *et al*. (2000). The transpiration rate and stomatal resistance showed definite correlation with the increase in net photosynthetic rate. Mori *et al*. (2001) reported that salicylic acid induced stomatal closure of *Vicia faba* by generation of O_2^- in epidermal peels.

Li Yue Ying and Chen Fen Yu (2001) observed an increase in chlorophyll content and photosynthetic rate in the rice plants sprayed with a growth retardant S 3307.

Tang *et al*. (1996) studied the relationships 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. Ethylene release rate was more under sterile condition in TGMS lines, and the application of amino vinyl glycine (AVG) reduced the ACC content and ERR and restored partially the pollen fertility in the CMS line. However, exogenous application of ethrel counteracted the effect of AVG (Liang *et al*., 1999). Shehata *et al*. (2000) reported that salicylic acid, applied as foliar sprays at 50 -–1000 ppm in cotton at the time of flowering, increased the endogenous hormones such as cytokinin, GA_3 and auxins, while decreased the ABA content. Shehata *et al*. (2001) observed the effects of acetyl salicylic acid sprayed at 20 and 40 ppm on maize, and found an increase in the endogenous IAA and zeatin riboside with a decrease in endogenous ABA content. Spraying mustard plants with salicylic acid (10 to 500 µM) significantly reduced both H_2O_2 and catalase activity below the control levels (Dat *et al*., 1998). Swain and Tripathy (1999) observed a sharp increase in IAA oxidase activity and sudden decrease in peroxidase and catalase activities, with a low concentration of coumarin (10^{-3} M). An increase in the NRase activity was observed in soyabeans sprayed with different concentrations of salicylic acid (Pramod Kumar *et al*., 1999).

Li Yue Ying and Chen Fen Yu (2001) reported a gradual decrease in the SOD activities in rice flag leaf with a spray of the plant growth retardant, S 3307. Sivakumar *et al*. (2001) studied the effect of foliar spray of salicylic acid @ 100 ppm on pearl millet and found that it increased the activities of NRase and IAA oxidase

activities. Thangaraj and Lakshmi Praba (2000) and Senthil (2001) reported that salicylic acid induced male sterility was found to be associated with an increased activity of IAA oxidase. Application of I_2 and I_3 (Pyridoxinone derivatives) decreased total nucleic acids, RNA in anthers, proteins and proline contents in rice variety Guangluai 4 and it was concluded that male sterility induced by I_2 and I_3 is closely related to deficiencies in nucleic acids, proteins and proline during anther development (Zhang *et al*., 1996). Treatments of chemical hybridizing agent "Bao Chungling" (BCL) caused decrease in RNA and DNA contents in leaves and spikelets of both cultivars (Liu *et al*., 1998). Barley plants treated with salicylic acid @ 100 µ mol l^{-1} to 1 m mol l^{-1} showed decrease in the levels of total soluble protein and concomitantly in the levels of Rubisco (Pancheva and Popova, 1998). Tian *et al*. (1999) studied the effect of ethylene on RNA and DNA in the leaves and panicles of male sterile rice and reported that the treatment with ethrel reduced these biochemical parameters. Tarraf and Ibrahim (1999) observed an increase in protein content with sprays of BR and uniconazole. Tarchevsky *et al*. (2001) found that exogenous application of phytohormones induced the appearance of ^{14}C label in a polypeptide with a molecular mass of 29 kD in control, while salicylic acid induced the synthesis of 19-kD polypeptide.

Xiao *et al*. (1996) reported that treating the CHA "Jinaolin" (0.5 kg ha^{-1}) at the immature cob stage in wheat caused a reduction in carbohydrate content and an increase in proline accumulation significantly. Experiment with foliar spray of salicylic acid (50 – 1000 ppm) generally decreased the total soluble sugars, although the effects varied with the time and application rate in cotton (Shehata *et al*., 2000). Spraying rice with ABA (15 mg/lit) increased the starch content and decreased the soluble sugars with high starch synthase activities (Yang Jianchang *et al*., 1999). Liu

et al. (1998) observed a decrease in soluble carbohydrates in the leaves and spikelets of TPGMSR lines Hinghong SI and Pei ai 64 S.

Application of I_2 and I_3 decreased the proline contents in rice variety Guangluai 4 and it was concluded that male sterility is closely related to deficiencies in proline during anther development (Zhang *et al*., 1996). Yang *et al*. (2000) reported an increase in proline content in detached rice leaves with exogenous application of ABA. Sanaa *et al*. (2001) reported an increase in free amino acid contents of fruits and seeds in *Phaseolus vulgaris* by salicylic acid sprays.

Shehata *et al*. (2001) reported a reduction in phenol content with the application of acetyl salicylic acid @ 20 and 40 ppm in the maize plants, while Sanaa *et al*. (2001) observed an increase in the phenolic compounds by salicylic acid sprays @ 2 and 5 mM concentrations on *Phaseolus vulgaris*. Thangaraj and Lakshmi Praba (2000) reported an accumulation of phenols in TGMS lines, when sprayed with salicylic acid at 800 ppm. Similar finding was also reported by Senthil (2001).

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

The male sterility was expressed at various stages of anther development with the arrest of normal pollen development occurring after separation of the microspores from the tetrads (Kurek *et al*., 2002). Feng Jiuhuan *et al*. (2001) has divided the developmental process of pollen into eight stages, namely microspore mother cell formation, microspore mother cell meiosis, early microspore stage, middle microspore, late microspore, early bicellular pollen, late bicellular pollen and mature pollen stage based on the morphological changes. Observations on the changes in anther microstructure of PGMS rice in LD and SD conditions showed that there were vessel elements in sieve tubes in vascular bundles of anther septa in SD, but not in

long day treated plants where vessel elements, sieve tubes and parenchyma cells developed poorly. 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 might reduce nutrient supply to developing microspores in LD treated anthers and this might be one of the reasons for sterility (Wang *et al*., 1992).

Chen *et al*. (1990) investigated the anther and pollen of Annong IS, 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 the pollen development having stopped at the monokaryon stage. 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 was absent. The CGMS line Zhenshan 97A had typical nuclear degenerative pollen abortion, while all the GMS lines were nuclear proliferative types, and it was suggested that this type of male sterility 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 58 S. 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 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 i.e., inner tapetum, middle endothecium and outer epidermis. The reason for pollen abortion might be due to incompatibility between nuclear and cytoplasmic genes. The results of Feng *et al*.

(2000) showed the abnormalities of microsporocytic development caused pollen abortion in TGMS line Pei ai 64 S. Lakshmi Praba (1998) and Senthil (2001) reported that the microtomy sections of male sterile anthers showed persistant tapetum, whereas in male fertile anthers tapetum degenerated with thickened endothecium and epidermal cells. Nayeem Pasha *et al*. (2000) reported that the degeneration of tapetum began just earlier to pollen grain stage. Such anthers had distorted tetrads and sterile pollen grains which caused sterility in Gerbera.

2.8.1. Anatomical studies in relation to selective sterility of the chemical / female fertility

A method of inducing male sterility, leaving the female organ fertile, by a simple chemical treatment would be of great importance for the production of hybrid seed, where the costs of emasculation and pollination by hand are prohibitive to the production of hybrid seed on a large commercial scale, or where this method is impossible for tehcnical reasons, such as, smallness of reproductive organs (McRae, 1985). Chemical emasculators, despite many studies, have been least used in practical hybrid breeding programme. If a potential chemical is found and its application is optimised to realise desired level of selective male sterility, several of the advantages of chemical hybridising agent can be made use of for a successful two line breeding (Ladislav Dotlacil and Marie Apltauerova, 1978). When sprayed on the rice plants at specific developmental stages, a gametocide or chemical hybridizing agent can emasculate the plants, thus resulting in male sterility while maintaining normal female fertility (Lasa and Bosemark, 1993). Luo Qiong and Zhu Lihuang (2002) have reported that rice floral organs will provide the basis for grain formation. The stigma of *O. sativa* is typically dry and plumose. The pistil is bifurcated just above the ovary. The distal parts of the two main branches are densely covered by multicellular multiseriate papillae, and the cells of the transmitting tissue are polygonal, narrow and elongated (Ciampolini *et al*., 2001). Besides synchronization of flowering

between male and female parents and effective transfer of pollen from male to female parent, prolonged receptivity of stigma and viability of pollen are desirable characters to enhance seed set in CMS lines (Mankar and Singhal, 1997).

The pollen grain started germination soon after coming in contact with the stigma (Kihara and Hori, 1966). Selvanathan and Khanna (1989) reported that the tube growth started immediately after pollination through the papillate stigmatic hairs, and the growth rates differed between crosses while the germination of pollen is directly correlated to seed set. Namai (1987) reported that the tip of the pollen tube penetrated into the embryo sac through a synergid, then it burst and gave off the generative nuclei, one of which fertilized the egg cell and others fused with polar nuclei. Satake and Koike (1983) reported that cool temperature at the flowering stage prevented germination of pollen grains on the stigma and caused sterility. Hattori and Wada (1996) observed conditions of pollen tube elongation on the stigmatic hair and the portion of the style through which the pollen tube passed. Pollen tube behaviour in the ovary was also studied. They observed a narrow space between the outer wall of the ovule and the ovary wall, except at the dorsal portion of the locule, where the pollen tube passed through the micropyle. Matsui *et al*. (2001) has reported that the anthers of rice showed floret fertility even when the temperature was high because of the well developed cavities for dehiscence. Thicker locule walls enabled easy rupture of the septa in response to swelling of pollen grains, which is the driving force to open the theca as the septum ruptures.

Table 1. TGMS rice lines reported in India

TGMS line	Source
TS 1	Mutant from BG-850-1
TS 2	Mutant from BR-1728-26-1-1-5
TS 3	Mutant from IR 20-1579-1633-55-26-42
TS 4	Mutant from RP-2095-5-7-34
TS 5	Mutant from RP-2095-5-7-344
TS 6	Mutant from RP-2161-106-1-1
TS 7	BIC, F2 of IR 628292A / O. nivara / IR 62829 B
TS 8	Mutant from ASD 16
TS 9	Mutant from IR 50
TS 10	Mutant from C12 japonica purple
TS 11	Mutant from C12 japonica purple
TS 15	Mutant from IR 32364-20-1-3-2
TS 16	Selection from Norin PL.12/IR 56 (IR 68945)
TS 18	Selection from Norin PL.12/IR 36 (IR 68945)
TS 32	Selection from C 41
TS 33	Selection from C 42
TS 35	Selection from C 44
TS 36	Selection from C 45
SM 3	Variety Prana
SM 5	Germplasm
JP 2	Breeding line of unknown cross
JP 8-IA 12	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
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 / IR 47686-17-2
IR 68292	**Selection from Norin PL 12 / IR 47686-17-2**

(Latha, 2001)

Fig. 2. Pattern of fertility changes in relation to temperature in TGMS lines

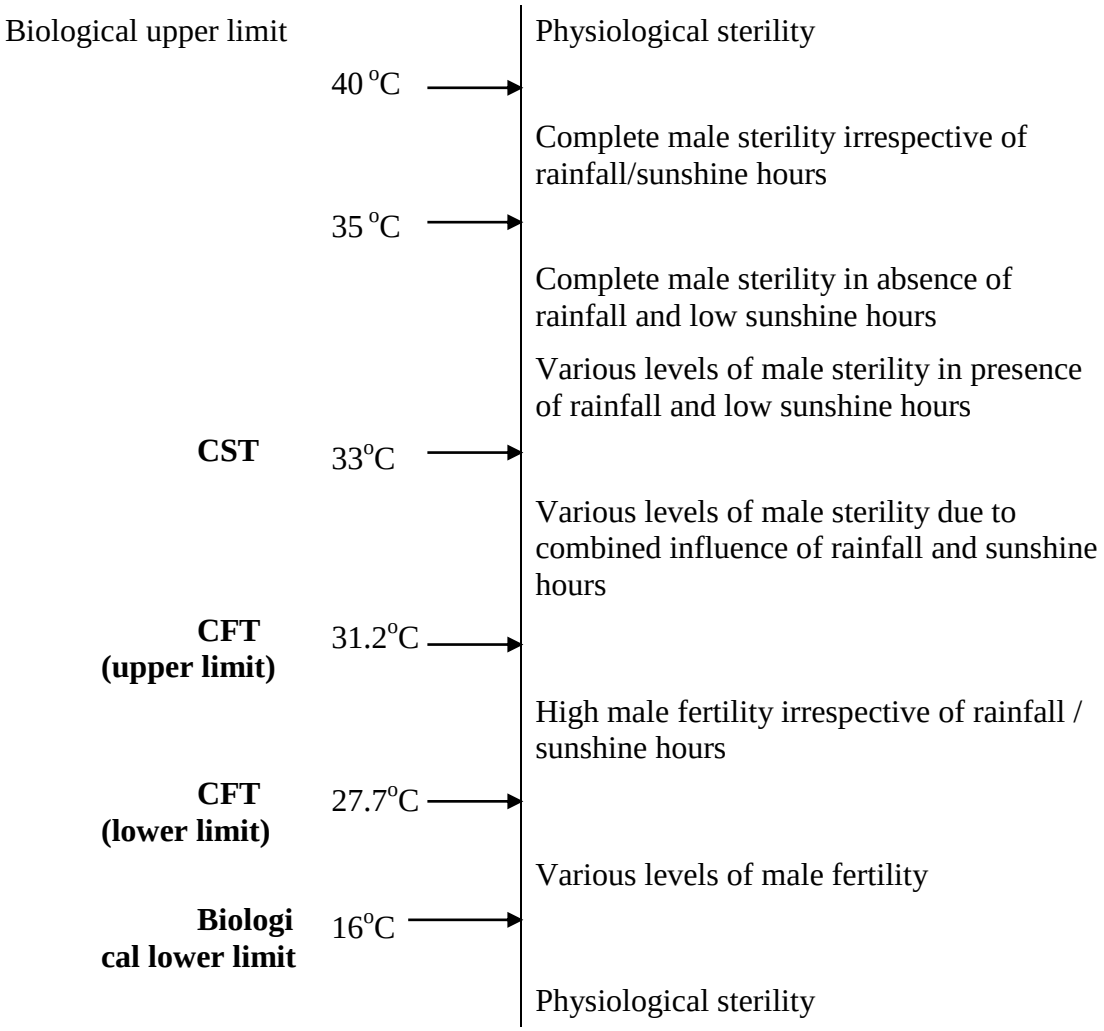

Table 2. Critical temperature for fertility alteration in different TGMS rice lines

TGMS line	Critical temperature	Reference
5460 S	29.5°C	Sun et al. (1989)
	28 – 29°C	Cheng *et al*. (1990)
	28.5°C	Sun *et al*. (1993)
Annong IS, W 6154 S	24.0 °C	Wu and Yin (1992)
Hennong IS	27.0 °C	Wu and Yin (1992)
W 8013 S	24.4 °C	Zhang and Lu (1992)
Norin PL 12	25 °C	Zhang *et al*. (1993)
R 59 TS	24.0 °C	Yang *et al*. (1990)
W 6154 S, W 8013 S	24.0 – 27.0 °C	Zhang *et al*. (1994)
Pei ai 64 S	21.0 – 24.0°C	Zhang *et al*. (1994)
KS – 14	21.0 – 24.0 °C	Zhang *et al*. (1994)
26 Zhaizao S (RTGMS)	23.0 °C	Shen *et al*. (1994)
T 436 S	26.1 °C	Dong *et al*. (1995)
SM 3	33.9 °C	Ali *et al*. (1995)
	20.0 – 24.0 °C	
SM 5	32.3 °C	Ali *et al*. (1995)
	20.0 – 24.0 °C	
SA 2	31.7 °C	Ali *et al*. (1995)
	20.0 °C	
JP 8-1A-12	30.9 °C	Ali *et al*. (1995)
	20.0 °C	
JP 2	33.9 °C	Ali *et al*. (1995)
	26.0 °C	

F 61	30.9 °C	Ali *et al*. (1995)
	24.0 °C	
Hennong 3S	27.0 °C	Gao *et al*. (1996)
IR 68 949	30.7 – 35.5 °C	Lu *et al*. (1996)
IR 32364	22.0 – 24.6 °C	

Table 2. (Contd.)

TGMS line	Critical temperature	Reference
68949-5-31-34	27.1 – 28.4 °C	Vijayakumar *et al*. (1996)
54603	33.0°C 25.0°C	Xiao *et al*. (1996)
TS 10	34.8 °C 32.5 °C	Elsy (1997)
TS 16	31.2 °C 30.7 °C	Elsy (1997)
TS 18	33.0 °C 31.2 °C	Elsy (1997)
UPRI 95-140 TGMS	28.7 °C 26.2 °C	Pandey *et al*. (1998)

Table 3. Critical stage of fertility alteration in different PGMS / TGMS rice lines

TGMS line	Critical stage	Reference
Nongken 58 S	Secondary rachis and spikelet primordia differentiation to PMC formation	Wan and Deng (1990)
Nongken 58 S	Stamen and pistil primordia differentiation	Wang *et al*. (1990)
5460 S	Meiosis of PMC	Cheng *et al*. (1990); Yang (1990)
	Stamen and pistil primordia differentiation to meiosis of PMC	Zhang *et al*. (1992)
	PMC formation to meiosis of PMC	Zhang and Lu (1992)
	PMC formation to late uninucleate stage	Sun *et al*. (1993)
TGMS IV-2	PMC in post metaphase to uninucleate stage	Zeng *et al*. (1992)
W 6154 S	PMC to single nuclear pollen stage	Zhang *et al*. (1994)
SA 2, JP 2, F 61, SM 5, SM 3	Stamen and pistil primordia differentiation	Ali *et al*. (1995)
GD 2S	Secondary rachis differentiation and spikelet primordia differentiation	Wang *et al*. (1997)
Norin PL 12	Stamen and pistil primordia differentiation to PMC formation	Borkakati and Virmani (1997)
	22 days before heading	Maruyama *et al*. (1990)
IR 32364 TGMS	Stamen and pistil primordia differentiation	Borkakati and Virmani (1997)
Annong IS	Stamen and pistil formation to meiosis of PMC	Liu *et al*. (1997)
Annong 810 S	Stamen and pistil formation	Liu *et al*. (1997)
TS 10, TS 16, TS 18	Secondary branch primordia differentiation to filling stage of pollen	Elsy (1997)
TS 16, TS 18	10 to 25 days before heading	Lakshmi Praba and Thangaraj (1999)
TS 16, TS 18	Secondary rachis branch primordia differentiation to early meiosis of pollen mother cell	Roystephen and Thangaraj (2000)

CHAPTER III

MATERIALS AND METHODS

The present investigation was carried out to understand the physiological and biochemical basis for sterility/fertility induction in Thermosensitive Genic Male Sterile (TGMS) rice, TS 29 with chemicals and growth regulators by laying out field trials at Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore, and by conducting laboratory experiments and Phytotron studies at the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore during 2000 – 2003. A detailed account of the materials used and methodologies followed in different experiments are presented in this chapter.

The details about the planting materials used in this study are furnished below. TS 29 rice line was identified as promising one with complete sterility under high temperature and with more than 50 per cent fertility under low temperature condition. The line was found to have stable sterile phase with 100 per cent pollen sterility for more than 50 days during high temperature season in Coimbatore (April to June). The line reverted to fertility during low temperature season (November to January).

CO 46 is one of the leading variety in Tamil Nadu with the parentage of T 7 x IR 20. The duration of the variety varies from 110 – 120 days. The variety is resistant to BPH, and the grain is chacterized with long cylinder white rice.

3.1. Experiment I. Identifying critical sterility temperature

The seeds of TS 29 were obtained from Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore. This formed the basic material for the study.

The TS 29 line was 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 4.

Table 4. Properties of soil used for pot culture experiment

Mechanical composition	
Coarse sand (%)	15.4
Fine sand (%)	34.6
Silt (%)	16.2
Clay (%)	33.8
Texture	Clay loam
Physico chemical properties	
pH	7.9
EC (d Sm^{-1})	0.35
CEC (meq 100 g^{-1})	38.5
Nutrient status	
Available N (kg ha^{-1})	252.0
Available P_2O_5 (kg ha^{-1})	10.9
Available K_2O (kg ha^{-1})	536.7

Package of practices, as recommended in Crop Production Guide of TNAU for rice crop, were followed for maintenance of the crop. Three replications were maintained under each treatment.

The Phytotron (Conviron E15 – Conviron Products Company, Manitoba, Canada), available in the Department of Crop Physiology was used in this experiment. The temperature regimes maintained for various treatments, in the growth chamber to get the required daily mean temperature, are given in Table 5.

3.1.1. Description of the Phytotron

The internal dimension of the growth chamber (inside space) was 6.0 x 2.5 x 5.0 ft (1 x b x h). 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^{o}C to 50^{o}C with an accuracy of 1^{o}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. Humidity can be adjusted from 20% RH to 95% RH. For humidity maintenance, distilled and demineralised water was supplied to avoid any salt deposition inside. For getting a constant current, the Phytotron was connected with 10 KVa Stabilizer. The parameters namely, light intensity, temperature and 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 in temperature from the set temperature was indicated with an alarm facility.

Treatments – The light duration of the chamber was adjusted to short day condition (10 hrs/day) to avoid any possible influence of long days in inducing pollen sterility. Light intensity of 400 to 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 25 days before flowering, and kept upto 5 days before heading (20 days duration). Pollen sterility behaviour of these plants were observed at the time of flowering. Five florets were collected from each panicle and anthers of these florets were squashed and the pollen were observed under microscope after staining with iodine – potassium iodide (I – KI) one per cent solution. Mean values of the percentage pollen sterility (unstained pollen grains) from these microscopic fields were considered for determining the critical sensitive temperature of the line.

3.2. Experiment II. Identifying critical stage of thermosensitivity

TS 29 line was exposed to sterility inducing temperature during different duration of the reproductive stage. The pollen sterility at flowering was noted for identifying the critical stage of thermosensitivity. Critical stage of thermosensitivity during panicle development and corresponding days before heading were assessed following the criteria as given in Table 6.

Table 6. 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 1 mm long and covered with white hairs	22 – 26
IV	6-7	Appearance of glumes, differentiation of stamen and pistil, young panicle about 0.5 to 1 cm long	19 – 22
V	3	Formation of pollen mother cell, floret about 1-3 mm long, young panicle 1.5-6 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)

TS 29 plants were grown in plastic pots and maintained in Glass house during winter season till they were transferred to the Phytotron. Three replications were maintained.

3.2.1. Experimental details

Line used : TS 29

Period of treatment : 3.10.2002 to 13.12.2002

Treatments [days before heading (DBH)]

T_1 : 0 – 30 DBH
T_2 : 0 – 20 DBH
T_3 : 0 – 10 DBH
T_4 : 5 – 30 DBH
T_5 : 5 – 25 DBH
T_6 : 5 – 10 DBH
T_7 : 10 – 30 DBH
T_8 : 10 – 25 DBH
T_9 : 10 – 20 DBH
T_{10} : 15 – 30 DBH
T_{11} : 15 – 20 DBH
T_{12} : 20 – 30 DBH

Replication : Three

Design : CRD

The Phytotron was set to an average temperature of 29^{o}C. Mean of the pollen sterility data were used to identify the critical stage of thermosensitivity.

3.3. Experiment III. Physiological and biochemical changes under pollen fertile and sterile conditions

The TS 29 line along with a known thermoinsensitive variety, CO 46 formed the basic material for the 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. The plants were kept inside Phytotron up to stage VII of panicle development and physiological and biochemical analysis were done

immediately after stage VII. The temperature treatment, given in the Phytotron to imitate the diurnal variation of summer season for induction of male sterility, is given below.

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

3.3.1. Experimental details

Lines used : TS 29 and CO 46

Treatments : T_1 – TS 29 – Glass house (Fertile pollen)
T_2 – TS 29 – Phytotron (Sterile pollen)
T_3 – CO 46 – Glass house
T_4 – CO 46 - Phytotron

Replication : Five
Design : CRD

Sowing date was adjusted, so that, the TS 29 line and CO 46 came to panicle initiation stage during the 1st week of December, 2001. Thus, the sowing was taken up on 10th October, 2001. The mean maximum and minimum temperatures for December and January were 28.6 and 19.9^{o}C respectively (Table 7).

3.3.2. IAA content

Quantitative determination of IAA content was done by indirect ELISA method as per the procedure of Anitha *et al*. (1996).

Two grams of the sample was extracted in ice-chilled 80 per cent methanol and the methanol was evaporated under vacuum to leave an aqueous aliquot.

i) Coating the ELISA plate with antigen (IAA-casein conjugate)

Antigen was diluted, using coating buffer of 100 ng per 200 µl (0.05 M, pH 9.5 containing Na_2CO_3 1.59 g, $NaHCO_3$ 2.93 g, sodium azide (NaN_3) 0.2 g in one litre water) and 200 µl of it was added to each well. The plate was incubated at 4^oC overnight. After the incubation, the contents were discarded and washed thrice with washing buffer (PBS with Tween 20, pH 7.4 containing NaCl 8 g, KCl 0.2 g, KH_2PO_4 0.2 g Na_2HPO_4 1.15 g, Tween 20 0.5 ml in one litre of water) at three minutes interval.

ii) Blocking the non-specific sites in the wells

The wells were fitted with blocking buffer pH 7.4 (casein 1 g and washing buffer 100 ml), and the plate was incubated at 37^oC for 90 minutes. The wells were then washed thrice with washing buffer as above.

iii) Addition of primary antibody

200 µl of primary antibody diluted with dilution buffer, was added to the wells and the plate was incubated at 37^oC for three hours to allow the IAA antibodies to bind to the IAA molecules, bound to each well. The wells were washed thrice with washing buffer as explained earlier.

iv) Addition of secondary antibody

Secondary antibody (goat anti-rabbit antibody) of 200 µl, diluted to 1:1500 with dilution buffer, was added to the wells. The plate was incubated at 37^oC for two hours and washed thrice with washing buffer.

v) Detection

The substrate for alkaline phosphatase and para-nitrophenol phosphate (PNPP) was prepared by coating buffer without azide (1 ml ml^{-1}) and 200 µl was added to each well. The plate was incubated at 37^{o}C for colour development (15 min). Reaction was stopped by adding 5 µl of 5 N KOH. PNPP, added to two empty wells, was taken as control. The absorbance was read at 405 nm using ELISA reader.

Construction of standard curve

In step three, instead of 200 µl of diluted serum, the IAA antibody was mixed with different concentrations of free IAA, diluted with Tris buffer saline, before being added to the coated microlitre well. Free IAA would bind to the specific antibodies in solution and prevent their subsequent binding by competitive inhibition to the IAA / casein attached to the plate. Then, 200 µl of diluted secondary antibody, having the attached chromogenic enzyme, was added to wells which binds to IAA antibody. The intensity of final colour in each well was inversely proportional to the concentration of free IAA added to the well.

3.3.3. ABA content

Quantitative determination of ABA content was done by indirect ELISA method, as per the procedure of Kulkarni *et al*. (2000). The procedure of Wolf *et al*. (1990) was followed with minor modification. All extractions were carried out in dim, indirect light. Extracts were stored at 4^{o}C in the dark and analysed within two days. The frozen tissues (50 mg) were extracted by grinding and stirring in 25 ml of 90 per cent methanol containing 10 mg l^{-1} of BHT. Extraction was continued at 4^{o}C for 48 h. The clear extract was then passed through a Sep-Pak C-18 Cartridge (Millipore Corporation Milford, Mass, USA) to remove pigments and lipids from the extract. The methanol was removed by vacuum and the aqueous residue was partitioned three times against ethyl acetate at pH 3 to separate free ABA from its conjugates. The pooled ethyl acetate fractions were reduced to dryness under reduced pressure, and the residue was taken up in Tris buffer saline (TBS) (50 mM Tris, 150 mM NaCl, 1.0 mM $MgCl_2$, pH 7.8). Recovery levels were estimated by adding (±)

[^{3}H] ABA, to the methanolic extracts. Recovery of ABA after purification was 80-85 per cent.

ABA iummunoassay

Polyclonal antibodies were raised against the C_1 carboxyl site of (±) cis-trans ABA, adopting the procedure of Quarrie and Galfre (1985), after conjugating it to a carrier protein bovine serum albumin. Presence of antibodies to the hapten ABA was checked by standard methods. Cross reactions of the C1 antibodies to the naturally occurring conjugate ABA-glucose ester has been shown to be high in previous studies (Weiler, 1982; Ryu and Li, 1994). Therefore, a meticulous fractionation procedure was followed to remove ABA conjugates from the extracts, following the procedure described by Ryu and Li (1994).

Ethyl acetate fractioning ensured that only free ABA remained in the sample, as the aqueous phase after partitioning containing the conjugates was discarded. However to validate the assay, two samples were compared for ABA content using ABA-Cl antibody, as well as, the commercially available monoclonal antibody from Idetek (a gift from Dr.Rana Munns, CSIRO, Australia). Using this antibody, indirect ELISA, modified after Weiler (1982), was adopted. Non specific binding was minimised by using one per cent casein in PBS-Tween in the blocking step. Each well received 0.2 ml of a 20 µg ml^{-1} IgG solution in 50 mM $NaHCO_3$ pH 9.6. The enzyme conjugate dilution was chosen so that, under assay conditions described, absorbance A_{405} = 1.0 for 100 per cent was obtained after one hour less than five per cent (equivalent to A_{405} = 0.05).

3.3.4. Ethylene release rate (ERR)

Young panicles at heading stage of sterile and fertile TS 29 line and of CO 46 were covered with 200 gauge thick polythene covers which were fitted with septum to draw the ethylene samples from the covers. Samples (2 mL) were drawn after 24

hours from the plastic covers with a gas tight hypodermic syringe. Ethylene was analysed with a Shimadzu GC-1 A gas chromatograph equipped with a flame ionization detector and an alumina column GS-Q (30 m x.053)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). For standard 10 µg (Z) of pure ethylene was injected into a 100 mL sealed conical flask. From that 2 mL of the standard was injected into the GLC to measure the ethylene peak height.

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

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

3.3.5. Gas exchange parameters

Gas exchange parameters namely, photosynthetic rate (µ mol CO_2 m^{-2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}) and diffusive resistance (s cm^{-1}) were recorded using an advanced portable CO_2 Gas Analyzer (model CI – 301 CID Inc., Made in USA). The readings were taken during 11:00 to 12:00 noon on clear sunny day, when the PAR was more than 1000 µ moles m^{-2} ℓ^{-1}. Second fully opened leaf from the top was 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 CO_2 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.

3.3.6. Hill reaction

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

3.3.6.1. Isolation of chloroplast

One gram 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 five minutes. The supernatant was discarded. The pellet was resuspended with isolation medium and centrifuged at 7000 rpm for five min. The supernatant was discarded and the pellet was resuspended in one ml of resuspension buffer and stored in ice and used within two hours for estimation. The whole procedure was carried out at about 4^{o}C temperature.

Reagents

A.	Isolation medium	330 mM	sorbitol
		5 mM	$MgCl_2$
		10 mM	NaCl
		2 mM	sodium ascorbate
B.	Resuspension medium	330 mM	sorbitol
		2 mM	EDTA
		5 mM	$MgCl_2$
		10 mM	NaCl
		20 mM	Tris-HCl (pH 7.5)
C.	Reaction mixture/buffer	20 mM	Tris-HCl (pH 7.5)
		10 mM	NaCl
		10 mM	$MgCl_2$
		100 mM	sucrose
		150 mM	NH_4Cl
D.	Dye	20 mM	DCIP

3.3.6.2. Estimation of Hill reaction

The cuvette containing 2.87 ml of reaction buffer, 0.02 ml of chloroplast suspension and 0.1 ml of NH_4Cl (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 and 20 mM DCIP was added and the initial absorbance was measured. The cuvette was then taken out and illuminated with light from a 200 W bulb for 30 sec. 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 NH_4Cl and 0.01 ml DCIP (20 mM).

The total chlorophyll content of the chloroplast suspension was also estimated by dissolving 0.02 ml of chloroplast suspension in two ml of acetone 80 per cent and measuring absorbance at 645 nm and 663 nm. The rate of photosynthetic electron transport was expressed as micromoles of DCIP reduced per milligram chlorophyll per hour.

3.3.7. Nucleic acid (DNA and RNA) contents

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

3.3.8. Carbohydrate fractions

3.3.8.1. Reducing sugars and total sugars

Reducing sugars and total sugars were estimated from the ethanol extract of leaves following the method of Somogyi (1952) and expressed as mg g^{-1} FW.

3.3.8.2. Starch content

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.

3.3.8.3. Total carbohydrates

Total carbohydrates 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.

3.3.9. Biochemical constituents

3.3.9.1. Soluble protein content

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

3.3.9.2. Proline content

Proline was estimated by selective extraction with three 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.

3.3.9.3. Total phenols content

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

3.3.9.4. Total free amino acids

Total free amino acids content was estimated from the ethanol extract by reaction with ninhydrin as per the method of Sadasivam and Manickam (1996) and expressed as mg g^{-1} FW

3.3.9.5. Ascorbic acid content

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

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

3.3.10. Enzyme activities

3.3.10.1. IAA oxidase

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

One gram of leaf sample was extracted in cold distilled water and to a known volume of the extract, added one ml of phosphate buffer (pH 6.6), one ml of auxin and one ml of distilled water and kept for incubation on a water bath at 25^{o}C for one hour. The residual auxin, available in the reaction mixture, was estimated by adding eight ml of Garden-Weber reagent. The intensity of pink colour developed was measured at 540 nm and the enzyme activity was expressed as µ moles of unoxidised auxin g^{-1} hr^{-1}.

3.3.10.2. Nitrate reductase

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

3.3.10.3. Ascorbic acid oxidase (AAO)

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

One gram of leaf samples was macerated and extracted with 0.1 M phosphate buffer (pH 6.5). A known volume of extract was added to the experimental cuvette containing three ml of substrate solution with ascorbic acid. The change in absorbance at 265 nm for every 30 seconds interval was measured for five 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 at 0^o to 5^oC.

3.3.10.4. Glutamate dehydrogenase (GDH)

GDH activity was determined by adopting the method of Sadasivam and Manickam (1996).

One gram of the leaf tissue was macerated and extracted with five ml of 100 mM phosphate buffer pH 7.5 containing one mM disodium EDTA, one mM dithioerythritol and one per cent polyvinyl pyrolidone (PVP) and centrifuged at 10,000 rpm for 30 min at 4^oC. The supernatant was used for enzyme assay.

Materials

Potassium phosphate buffer (pH 7.0)

2 – oxoglutarate (0.1 M : 14.6 g l^{-1} distilled water)

NH_4Cl (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_4Cl	0.5
4.	NADH	0.12
5.	Enzyme extract	0.2
6.	Water	8

To the blank, 0.3 ml of water was added instead of 2-oxoglutarate. The reaction mixture was incubated at 37^{o}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 co-efficient. Activities are expressed as nanomole NADH oxidised per min per ml enzyme source.

3.3.11. Active oxygen species scavenging enzymes

3.3.11.1. Catalase

Catalase activity was determined following the method of Luck (1974).

One gram of the sample was macerated and extracted in 0.067 M phosphate buffer (pH 7.0). A known volume of the extract was added to the experimental cuvette containing three ml $H_2O_2 – PO_4$ 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 g^{-1} tissue. All the operations were carried out at 0 – 5°C.

3.3.11.2. Peroxidase

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

One gram of leaf was macerated and extracted in 0.1 M phosphate buffer (pH 7.0). A known volume of the extract was added to an experimental cuvette containing three 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°C.

3.3.11.3. Superoxide dismutase

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

	Reagent stock	Working solution	Total volume
1.	Riboflavin 200 µM (100 x) 3.8 mg/50 ml	2 µM	30 µl
2.	Buffer 250 mM (5 x) 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 (100 x) 0.3722 g/100 ml	0.1 mM	30 µl
5.	NBT 750 µM (100 x) 40.8 mg/5 ml	75 µM	30 µl
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

$$\frac{x - y}{x} \times 100 = 2\% \text{ inhibition}$$

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

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

1000 µl yield is A/50 x 1000 = B unit

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

3.3.12. Nutrient analysis

The third leaf from apex at Stage VII of panicle development and young panicles at booting stage were analysed for the following nutrients.

3.3.12.1. Nitrogen content

Total nitrogen content was estimated from the triple acid extract (nitric acid: sulphuric acid: perchloric acid 9:3:2) by microkjeldhal method as determined by Humphries (1956) and the content was expressed as percentage.

3.3.12.2. Potassium content

Potassium was determined in triple acid extract using flame photometer Type 21, Systronics, Ahmedabad and expressed as percentage on dry weight basis (Jackson, 1962).

3.3.12.3. Calcium content

Calcium content of leaves and young panicles was analysed by versanate titration method proposed by Piper (1966) and expressed in µg g^{-1} on dry weight basis.

3.3.12.4. 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 was expressed in µg g^{-1} of sample on dry weight basis.

3.3.12.5. Boron content

The content of boron in leaves and young panicles was estimated by the method proposed by Banuleas *et al*. (1992) using azomethane-H, and expressed in µg g^{-1} of sample on dry weight basis.

3.4. Experiment IV : Relative biochemical changes in TS 29 producing fertile and sterile pollens

TS 29 plants were grown in plastic pots in Glass house. The plants were transferred to the growth chamber when they were in stage III of panicle development. The plants were kept inside Phytotron up to stage VII of panicle development and various biochemical analysis were done at stage VII. The sowing dates and the temperature treatments given in the Phytotron, were same as in Experiment III.

3.4.1. Identification of specific amino acids by paper chromatography

Whatman No.1 chromatography paper of 46 x 57 cm size was spotted with 100 µl of samples and ascending chromatography was performed (Plummer, 1990) with a solvent saturated atmosphere. The solvent system of n-butanol: acetic acid: water (4:1:5) was used. The chromatographs were spotted by spraying 0.1 per cent ninhydrin reagent dissolved in acetone using known standards. The amino acid, present in the extract, was identified based on the Rf values of the standard amino acids.

Table 8. Rf values for standard amino acids.

Free aminoacids		Rf value
L-cysteine	-	7.2
L-histidine	-	13.5
L-leucine	-	9.8
DL-tyrosine	-	15.2
DL-isoleucine	-	11.8
L-lysine	-	8.7

3.4.2. Identification of individual phenols through HPLC (High Pressure Liquid Chromatography)

Extraction of leaf samples

Fractionation of phenolic compounds was done by a modified method of Oszmianski *et al*. (1988).

500 mg of leaf sample was extracted with 70 per cent aqueous acetone containing 0.1 per cent ascorbic acid. Acetone was removed by vacuum – rotary evaporation below 40^{o}C and the aqueous residue was washed with diethyl ether. The neutral aqueous fraction was loaded on a pre-conditioning neutral Sep Pak C18 cartridge. The cartridge was flushed with water and 0.01 M HCl, the eluate was collected and passed through into another cartridge that was pre-conditioned with

0.01 M HCl. The eluate from acidic Sep Pak that contained phenolic acids were collected. From the neutral Sep Pak, three fractions were collected. The lowest fraction was dried and redissolved in 250 ml with 70 per cent methanol and readed for HPLC analysis. Ultrasphere (ODS) column or C 18 (25 cm x 0.4 mm / 0.5 µm) was used in a Hitachi – HPLC L 6200 analyser. The phenol was detected by UV-detector at 254 mm wavelength. Methanol : Distilled water @ 1:1 was used as mobile phase (Boopathy, 1997). Standards were run at similar conditions. Based on the retention time of standard phenolic compounds and the area of normalisation, the compounds present in the samples were identified and analysed, using the formula.

$$\text{Quantity of phenols (mg g}^{-1}) = \frac{A_S}{A_{Std}} \times \frac{W_{Std}}{W_S} \times \frac{V_{ex}}{V_S} \times \text{error}$$

where,

A_S = Area of the sample

A_{Std} = Area of the standard

W_{Std} = Weight of the standard injected in ng (20 ng)

W_S = Weight of the sample in g (0.5 g)

V_{ex} = Volume of the final extract in ml (1 ml)

V_S = Quantity of the sample injected (20 ng)

Error = 1.2

Table 9. Retention time and the area of the standard phenols

Phenolic acids	RT (min)	Area
Benzoic acid	1.18	366325
Catechol	1.64	451432

Ferulic acid	1.22	382937
Pyrocatechol	1.57	409815
Salicylic acid	1.84	287392
Syringic acid	1.20	253518

3.4.3. Estimation of polyamines through HPLC

The polyamines in the leaf and young panicles of fertile and sterile TS 29 rice line at stage VII of panicle development were analysed by the benzoylation method as reported by Serrano *et al*. (1995). Extracts for polyamine analysis were prepared by homogenizing one g of plant sample (leaf and young panicles) in five ml of five per cent perchloric acid. The homogenate was then centrifuged for 30 min at 20,000 rpm and free polyamines were determined in the supernatant fraction. One ml of 4 M NaOH were mixed with one ml of perchloric acid extract and 10 ml of benzoyl chloride. After vortexing for 15S, the mixture was incubated for 20 min at room temperature. Then two ml of saturated NaCl was added and the benzoyl polyamines were extracted with two ml of cold diethyl ether centrifuged at 15,000 rpm for 20 minutes. Finally, one ml of ether phase was collected and evaporated to dryness under a stream of warm air, and redissolved in one ml methanol (HPLC grade). Derivatives were analyzed by HPLC using a HPLC – Hitachi L-6200 pump, LC-organizer D-2500 integrator. The elution system consisted of methanol : water (64:36 v/v) as solvent, run with a flow rate of 0.8 ml min^{-1}. The benzoyl-polyamines were eluted through reversed-phase column (ultrasphere – 150 mm x 4.6 mm) particle size five µm and detected by absorbance at 244 nm. A relative calibration procedure was used to determine the amounts of polyamines in samples using standard curves of putrescine, spermidine and cadaverine from sigma. Three extractions of polyamines were made from each sample and each one was quantified in duplicate using the formula.

$$\text{Quantity of polyamines } (\mu\text{mol } g^{-1}) = \frac{A_S}{A_{Std}} \times \frac{W_{Std}}{W_S} \times \frac{V_{ex}}{V_S} \times \text{error}$$

where,

A_S = Area of the sample

A_{Std} = Area of the standard

W_{Std} = Weight of the standard injected in ng (20 ng)

W_S = Weight of the sample in g (1 g)

V_{ex} = Volume of the final extract in ml (1 ml)

V_S = Quantity of the sample injected (20 ng)

Error = 1.5

The retention time for the standards putrescine, spermidine and cadavarine were 2.18, 3.46 and 5.65 minutes respectively, and the area covered by putrescine, spermidine, and cadavarine were 9345, 11104 and 14734 per cent respectively.

3.5. Experiment V: Induction of sterility in TS 29 using chemicals and growth regulators

An experiment was conducted to study the effect of various plant growth regulators and chemicals in inducing sterility at Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore during two seasons, Rabi 2001-2002. (partial sterility phase of TS 29 line),(Plate 5) and Kharif 2002 (partial sterility phase of TS 29 line),(Plate 7). The stable line namely, TS 29 was used in this experiment. The chemicals and plant growth regulators were tried with different concentrations, sprayed at two stages of panicle development. This was done to identify an effective chemical and their concentration to induce 100 per cent pollen sterility. Pollen sterility before anthesis was estimated by I-KI staining.

3.5.1. Details of the soils of the experimental sites were

Mechanical composition	-	Clayey
pH	-	8.0
EC (d Sm^{-1})	-	0.38

Nutrient status

$KMnO_4$-N	-	166 kg ha^{-1}
Olsen-P	-	11 kg ha^{-1}
NH_4 OAC-K	-	394 kg ha^{-1}
Organic carbon	-	6.9 g kg^{-1}

3.5.2. Experimental details

Treatments : 5

T_1	-	Control
T_2	-	Salicylic acid – 800 ppm
T_3	-	Salicylic acid – 1000 ppm
T_4	-	Ethrel – 800 ppm
T_5	-	Maleic hydrazide – 2000 ppm

Replication : Four

Design : RBD

Stages of spray

1^{st} spray	:	Stage III of panicle development
2^{nd} spray	:	Stage V of panicle development

3.5.3. Details of the first season field trial (Rabi, 2001 – 2002)

14^{th} December to 25^{th} April

Date of sowing	:	14.12.2001
Date of transplanting	:	11.1.2002
Date of first spraying	:	6.2.2002
Date of second spraying	:	16.2.2002
Spacing	:	10 x 15 cm
Plot size	:	3 x 4 m
Type of nursery	:	Raised bed
Period when the pollens were observed for sterility	:	2.3.2002 to 15.3.2002
Date of sowing of 'R' line (MDU 5)	:	14.12.2001
Date of transplanting	:	11.01.2002
Transplanting ratio of A:R line	:	8:2

3.5.4. Details of the second season field trial (Kharif 2002)

12^{th} July to 22^{nd} November

Date of sowing	:	12.7.02
Date of transplanting	:	8.8.02
Date of first spraying	:	6.9.02
Date of second spraying	:	15.9.02
Plot size	:	3 x 4m

Spacing	:	10 x 15 cm
Period when the pollens were observed for sterility	:	30.9.02 to 13.10.02
Date of sowing of 'R' line (MDU 5)	:	12.07.02
Date of transplanting of 'R' line	:	8.8.02
Transplanting ratio of A:R line	:	8:2

3.5.5. Weather during the experimental period

The meteorological data, recorded at Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore, where the field experiments were conducted during first season (Rabi, 2001-2002) and second season (Kharif, 2002) are furnished in Table 10 and Table 11.

Weather observed during the first season (Rabi, 2001-2002)

During panicle development (0 – 30 DBH) the maximum temperature ranged from 29.8^{o}C to 33.2^{o}C and the minimum temperature ranged from 17.6^{o}C to 21.0^{o}C. The mean temperature during this period ranged between 24.8^{o}C and 26.2^{o}C.

Weather observed during the second season (Kharif, 2002)

During panicle development (0 – 30 DBH) the maximum temperature ranged from 28.7^{o}C to 32.8^{o}C and the minimum temperature ranged from 21.1^{o}C to 23.3^{o}C. The mean temperature during this period ranged between 25.9^{o}C and 26.9^{o}C.

3.5.6. Observations recorded

3.5.6.1. Per cent pollen sterility

Per cent pollen sterility was observed before anthesis. Pollens were stained with I-KI dye, 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.

3.5.6.2. Per cent crossed seed set

Clipping method: After the confirmation of 100 per cent sterility, the spikelets were opened by clipping method and dusted with pollen grains collected from the pollinator (MDU 5), and covered by white crossing covers. After maturity, percent crossed seed setting was worked out.

Rope pulling method: This method is commonly used in hybrid seed production in rice. Here, the panicles of fertile lines (i.e.) 'R' line were pulled by ropes to shed their pollen grains over the spikelets of male sterile TS 29 line. After maturity, per cent crossed seed setting was worked out.

3.5.6.3. Biochemical parameters

Leaf samples were collected from control and treated plants five days after first treatment (stage III), five days after second treatment (stage V) of panicle development and flowering stages for biochemical analysis of

i) Starch content
ii) Total carbohydrates content
iii) Soluble protein content
iv) Proline content
v) Total phenols content
vi) Individual phenols (analysed after giving second chemical spray during panicle development in second season crop)

vii) Total free amino acids

viii) Ethylene release rates from emerging panicles, estimated during the second season crop

ix) IAA oxidase activity

x) Nitrate Reductase activity

xi) Ascorbic acid oxidase activity

xii) Catalase activity

xiii) Peroxidase activity

xiv) Superoxide dismutase activity

The contents of protein, carbohydrates, phenols, proline, amino acids, all enzyme activities, identification of individual phenols and ethylene release rates were estimated as per the procedures described in the Experiments III and IV.

3.6. Experiment VI : Chemical manipulation of sterility using phenols

3.6.1. Glass house condition

Along with salicylic acid, other related phenols were tried on the induction of male sterility in TS 29 line, at the Glass house of Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore.

3.6.1.1. Experimental details

Date of sowing	:	2.4.02
Date of transplanting	:	25.4.02
No. of pots maintained	:	27
No. of treatments	:	9
No. of replications	:	3
Design	:	RBD

Date of 1st spray : 31.5.02

Date of 2nd spray : 10.06.02

Period when pollens were observed for sterility : 25.6.02 to 7.7.02

Period when spikelets were observed for sterility : 1.8.02 to 10.8.02

3.6.1.2. Treatmental details

T_1 - Control

T_2 - Salicylic acid (1000 ppm)

T_3 - Ferulic acid (1000 ppm)

T_4 - Benzoic acid (1000 ppm)

T_5 - Chlorogenic acid (1000 ppm)

T_6 - Cinnamic acid (1000 ppm)

T_7 - Syringic acid (1000 ppm)

T_8 - Catechol (1000 ppm)

T_9 - Pyrocatechol (1000 ppm)

Stages of spray

1st spray : Stage III of panicle development

2nd spray : Stage V of panicle development

3.6.1.3. Observations recorded

1. Pollen sterility
2. Spikelet sterility : Total number of chaffy spikelets (without seeds and white in colour) were counted as sterile

3.6.2. Field trial

The promising phenols that were found to induce pollen sterility under Glass house condition, namely salicylic acid, cinnamic acid, catechol and pyrocatechol were tried on TS 29 at field level at Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore.

3.6.2.1. Experimental details

Date of sowing	:	12.07.02
Date of transplanting	:	8.8.02
No. of treatments	:	5
No. of replications	:	4
Design	:	RBD

3.6.2.3. Treatmental details

T_1 - Control

T_2 - Salicylic acid (1000 ppm)

T_3 - Cinnamic acid (1000 ppm)

T_4 - Catechol (1000 ppm)

T_5 - Pyrocatechol (1000 ppm)

Stages of spray

1^{st} spray : Stage III of panicle development

2nd spray : Stage V of panicle development

3.6.2.4. Observations recorded

1. Pollen sterility
2. Spikelet sterility

3.6.2.5. Weather details

The layout of this experiment was done along with the second season crop raised for induction of male sterility using chemicals and growth regulators. So the weather data remains the same as for Kharif, 2002 experiment.

3.7. Experiment VII : Induction of fertility

This experiment was conducted in Rabi, 2001 – 2002 to study the effect of different growth regulators and hormones in increasing seed set of TS 29 line under field condition at Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore and at the Glass house, Department of Crop Physiology, TNAU, Coimbatore.

3.7.1. Experimental details

This field and Glass house trials were laid out along with the first season field trial for induction of sterility using chemicals and growth regulators (i.e.) during Rabi,2001 – 2002 (December 14th to April 25th), (Plate 6)

3.7.2. Treatmental details

T_1 - Control

T_2 - BR (1 ppm)

T_3 - NAA (50 ppm)

T_4 - IAA (50 ppm)

T_5 - Ascorbic acid (100 ppm)

No. of replication : 4

Design : RBD for field and CRD for Glass house trials.

Stages of spray

1^{st} spray : Stage III of panicle development

2^{nd} spray : Stage V of panicle development

3.7.3. Details of field trial for induction of fertility

Date of sowing	:	14.12.2001
Date of transplanting	:	11.1.2002
Date of first spraying	:	6.2.2002
Date of second spraying	:	16.2.2002
Plot size	:	3 x 4 m
Spacing	:	10 x 15 cm
Type of nursery	:	Raised bed
Period when the pollens were observed for fertility	:	2.3.2002 to 15.3.2002

3.7.4. Observations recorded

i) Pollen fertility percentage

ii) Seed set per cent

iii) Soluble protein content

iv) Starch content

v) Total carbohydrates content

vi) Proline content

vii) Total phenols content

viii) IAA oxidase activity

ix) Nitrate reductase activity

x) Catalase activity

xi) Peroxidase activity

xii) Superoxide dismutase activity

The procedures for analysis and observations for the above parameters are furnished in sections III and IV of this chapter.

3.8.1. Electrophoresis (SDS-PAGE)

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

A known quantity of leaf sample was homogenised in phosphate buffer (pH 7.0) in a prechilled pestile 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
Bisacrylamide	0.8 g
Water	100 ml

Separating gel buffer (pH 8.8)

1.875 m Tris-HCl	22.7 ml
Water	100 ml

Stacking gel buffer (pH 6.8)

0.6 M Tris-HCl	7.26 g
Water	100 ml

Polymerising agents

APS	0.5 g/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	2l

Sample loading buffer (5 x concentration)

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	10 ml

SDS 10% solution (Stored at room temperature)

Protein stain solution

Coomassie Brilliant Blue R 250	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 damped in an upright position on a gel casting unit with two 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

Degased for 3 – 5 min and added

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

The gel solution was poured into the chamber between glass plates carefully leaving four cm from the top and a layer of distilled water was added on top of the gel and allowed to polymerise for 30 – 60 min.

Stacking gel

Stock acrylamide	1.35 ml
Tris – HCl (pH 6.8)	1 ml
Water	7.5 ml
APS (5%)	50 µl
SDS (10%)	0.1 ml
TEMED	10 µl

After removing water from the gel, stacking gel was added, comb was placed and kept for polymerising (30 – 60 min). After polymerisation, the comb was taken out and the gel plate was removed from the casting unit to the electrophoresis apparatus. Concentration of the protein sample was adjusted to 50 – 200 µg into a volume of 25 – 50µl, by mixing with sample loading buffer and 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, and 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.

3.8.2. Isozyme analysis

500 mg of leaf sample was homogenised in one ml phosphate buffer (pH 7.0) in a prechilled 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 seven 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 refrigerator 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.

Peroxidase (POX) staining

The gel was incubated in a solution, containing benzidine 2.08 g, acetic acid 18 ml, H_2O_2 (3 per cent) 100 ml and water 80 ml. When the bright blue coloured bands appeared on the gel, the reaction was arrested by immersing the gel into a large volume of 0.67 per cent NaOH or seven per cent acetic acid solution for 10 min. The gel was photographed immediately as the colour was highly unstable.

Superoxide dismutase (SOD) staining (Beauchamp 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	-	100 ml

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

Esterase (EST) staining (Sadasivam and Manickam, 1996)

Staining solution

100 mM naphthyl acetate	-	1 ml
Fast blue 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^{o}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. The relative position of each band in the gel was drawn schematically as a zymogram. Vandenberg and Wissman (1982) described the norms for the nomenclature of isozymes. The same norm was followed in this study. The acronyms for peroxidase, esterase and superoxide dismutase were POX, EST and SOD respectively. The isozymes were given numbers corresponding to their relative mobility (Rm) related to the dye front, preceded by the standard acronym for the enzyme.

3.9. Morphological, anatomical and biochemical changes in anthers and pollens

3.9.1. Microscopic 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 (I-KI) dye (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.

3.9.2. Anther anatomy study through microtome sectioning

Anthers were collected before dehiscence from the TS 29 line, both from fertile and sterile plants grown under Glass house and Phytotron condition. The anthers fixed in FAA (Formalin : Acetic acid : Alcohol) fixative. The anthers were dehydrated, infiltered, embedded and transverse sections were taken by the method of Johansen (1940) using a Leica microtome (Model: JUNg RM 2045) and stained in Azocarmine-G followed by counter staining in a mixture of aniline blue, fast green FCF, Orange-G and phosphotungstic acid. This staining technique of Hamm (1966) was found to give good contrast staining of different cells and tissues in the anthers. The sections were mounted in Canada balsam and the anthers were observed under microscope.

3.9.3. Electrophoretic separation of anther proteins

Extraction of proteins

A known weight of fresh anthers was extracted in minimal volume of 0.1 M phosphate buffer (pH 7.0) and centrifuged at 10,000 rpm for seven min in a refrigerated centrifuge at 4^{o}C. The supernatant was collected and used for loading in electrophoresis.

Twelve per cent polycrylamide gel electrophoresis was done as per the procedure of Laemmli (1970). The composition of reagents used, method of casting, running of gel and staining procedures were followed as explained in the previous experiment.

3.10. Assessing the female fertility / selective sterility of the chemical by fluorescence microscope techniques

This technique was adopted to prove that the chemicals sprayed effected only selective sterility to male organs (i.e.) the pollens without affecting the female fertility. After confirmation of 100 per cent pollen sterility, induced by salicylic acid, the spikelets of TS 29 line were opened by clipping method and dusted with pollen grains collected from the pollinator (MDU 5) and covered with white crossing covers. Then, the ovaries collected from TS 29 line after four and eight hours were fixed in alcohol : acetic acid mixture in the ratio of 3:1 for 24 hours. After that, the ovaries were washed with distilled water for 3 to 5 times. NaOH of 8N was added to it and kept for four hours. Again three to five washings with distilled water were carried out. Aniline blue (0.1%) dissolved in 1 N K_3PO_4 was added and kept in dark overnight. Then, ovaries were mounted in 50 per cent glycerol and observed under fluorescence (Sitch, 1990).

Further confirmation of female fertility was done by taking microtome sections of the bottom of the ovary by adopting the method of Johansen (1940).

Statistical analysis

The data were analysed statistically for completely randomised design for Experiment I, II and III. The experiment V, viz., the induction of male sterility by chemicals and growth regulars involved two season field trials and hence, the data were analysed by pooled analysis. In experiment VI, completely randomised design was adopted for Glass house study, while in the field trial experiment for induction of

sterility using phenols and induction of fertility (Expt. VII), the data were analysed by randomised block design. Correlation studies of different physiological and biochemical parameters with pollen sterility of TS 29 line induced either by critical high sterility temperature condition or by salicylic acid were also carried out as per the method suggested by Webber and Moorthy (1952).

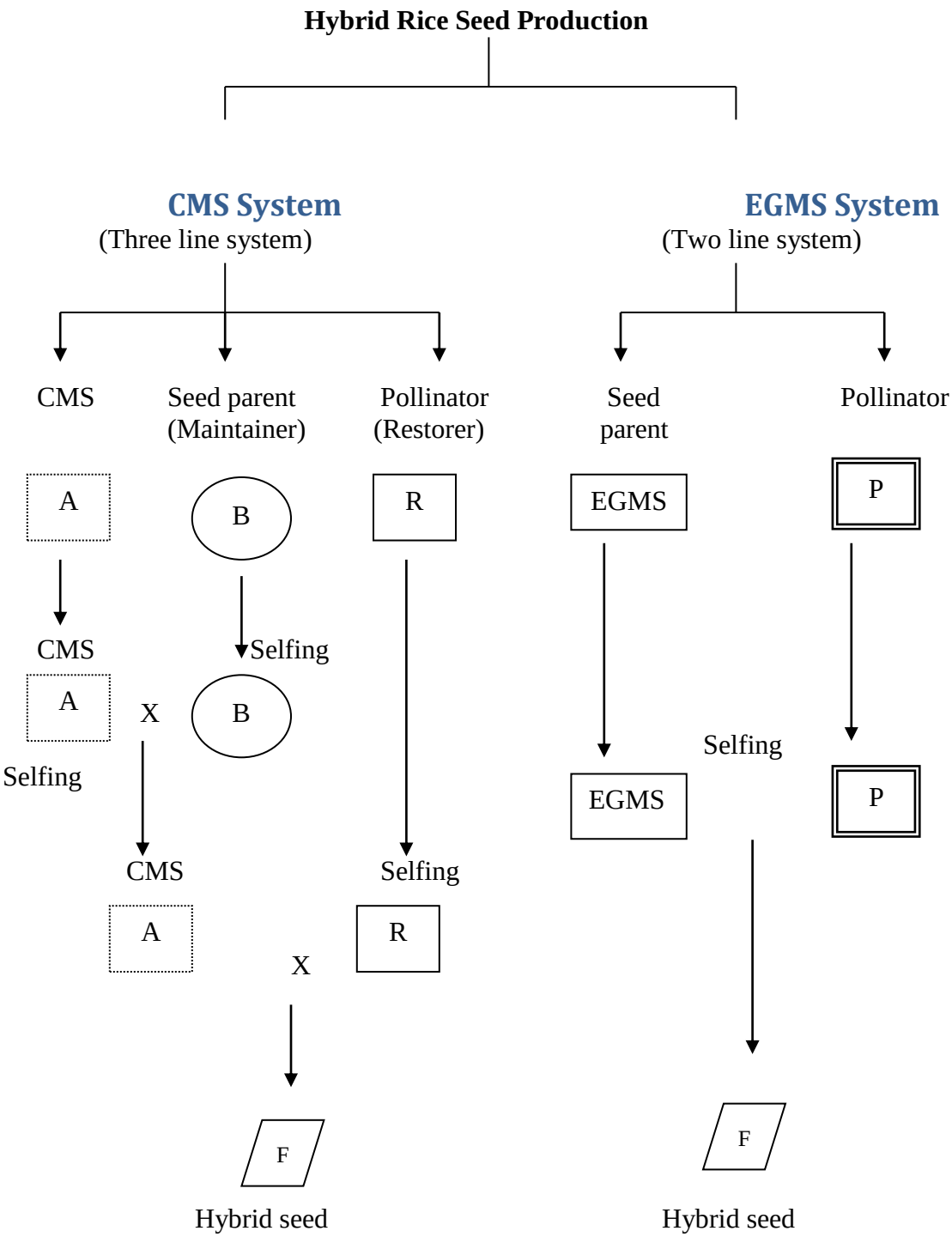

Fig.1. Comparison of hybrid rice seed production systems

Table 7. Glass house weather data for fertile TS 29 and CO 46 rice (10th October to 4th February)

Standard weeks of the months	Maximum Temp ^{o}C	Minimum Temp ^{o}C	Mean Temp (^{o}C)	RH (%)	Rainfall (mm)	Sunshine hours	Wind velocity (kmph)	Solar radiation (cal/cm^2/h)
October, 2001								
08-14	31.4	22.7	27.1	88	-	4.8	4.1	334.4
15-21	31.7	21.7	26.7	93	152.3	6.3	3.2	354.8
22-28	30.3	19.3	24.8	92	130.4	5.4	2.9	334.4
29-04	30.3	21.7	26.0	91	17.6	6.3	2.1	359.2
November, 2001								
05-11	30.4	22.2	26.3	93	30.9	5.3	1.6	352.4
12-18	29.6	22.1	25.9	91	48.5	4.7	2.2	308.4
19-25	28.3	20.9	24.6	92	34.1	7.6	3.2	383.3
26-02	28.7	18.0	23.4	91	8.3	6.9	2.7	364.6
December, 2001								
3-9	28.9	15.1	22.0	92	0.5	8.2	2.5	463.0
10-16	28.6	19.9	24.3	90	-	9.7	3.2	502.7
17-23	27.4	20.6	24.0	90	14.3	2.0	3.9	231.4
24-31	28.0	19.9	23.9	90	0.5	6.0	5.5	370.1
January, 02								
01-07	29.3	19.1	24.2	86	-	7.6	7.3	409.7
08-14	29.7	17.0	23.4	88	-	8.3	6.8	432.6
15-21	30.1	21.0	25.6	90	-	9.1	4.4	454.1
22-28	31.7	20.0	25.9	89	-	4.4	4.3	358.7
29-04	29.7	21.0	25.4	87	-	3.8	6.7	261.9
February, 02								
05-11	29.0	20.0	24.5	86	-	4.6	6.5	333.9

Table 10. Weekly weather data for the first season crop (Rabi, 2001-2002) (12th Dec. to 19th April)

Standard Weeks of the months	Maximum Temperature (°C)	Minimum Temperature (°C)	Mean Temperature (°C)	RH (%)	RF (mm)	Sunshine hours	Wind velocity (kmph)	Solar radiation (cal/cm²/d.)
December, 2001								
10-16	28.6	15.1	21.9	90	-	9.7	3.2	502.7
17-23	27.4	19.9	23.7	90	14.3	2.0	3.9	231.4
24-31	28.0	20.6	24.3	90	0.5	6.0	5.5	370.1
January, 2002								
1-7	29.3	19.9	24.6	86	-	7.6	7.3	409.7
8-14	29.7	19.1	24.4	88	-	8.3	6.8	432.6
15-21	30.1	17.0	23.6	90	-	9.1	4.4	454.1
22-28	31.7	21.0	26.4	89	-	4.4	4.3	358.7
29-04	29.7	20.0	24.9	87	-	3.8	6.7	261.9
February, 2002								
5-11	29.8	21.0	25.4	86	-	4.6	6.5	333.9
12-18	32.2	20.2	26.2	87	-	9.0	8.2	434.6
19-25	33.2	17.6	25.4	84	-	10.2	7.3	504.7
26-4	31.6	19.0	25.3	82	-	10.3	6.8	478.4
March, 2002								
5-11	30.1	19.4	24.8	78	-	10.6	7.0	460.9
12-18	30.3	21.7	26.0	84	28.0	9.5	7.7	424.6
19-25	34.6	22.1	28.4	88	4.0	8.7	7.1	435.6
26-01	30.3	24.0	27.3	86	37.2	8.4	5.0	415.9
April, 2002								
2-8	35.5	23.3	29.4	83	1.5	9.2	5.8	426.9
9-15	35.1	24.2	30.2	78	1.5	9.2	6.2	439.0
15-19	36.3	24.0	30.2	82	1.2	9.5	6.1	451.8

Table 11. Weekly weather data for the second season crop (Kharif, 2002) (12th July to 28th Nov.)

Standard Weeks of the months	Maximum Temperature (°C)	Minimum Temperature (°C)	Mean Temperature (°C)	RH (%)	RF (mm)	Sunshine hours	Wind velocity (kmph)	Solar radiation (cal/cm²/d.)
July, 2002								
9-15	32.5	22.5	27.5	81	-	6.9	17.5	395.2
16-22	33.0	23.1	28.1	81	1.0	5.4	6.4	371.3
23-29	32.6	21.9	27.3	88	1.5	6.9	5.6	379.3
30-5	31.1	22.6	26.9	86	20.4	5.0	15.4	340.0
August 2002								
6-12	29.2	23.0	26.1	72	5.9	1.9	24.4	288.6
13-19	30.5	21.6	26.1	76	10.5	6.0	23.7	307.6
20-26	30.8	21.6	26.2	87	-	7.1	11.9	401.2
27-2	30.5	22.1	26.3	92	27	6.6	6.9	373.4
September 2002								
3-9	28.7	23.1	25.9	89	-	8.6	16.9	431.8
10-16	32.8	21.1	26.9	88	2.6	9.5	11.6	441.2
17-23	30.0	21.8	25.9	89	16.3	7.8	7.5	399.6
24-30	30.1	22.0	26.1	87	1.0	9.1	7.0	420.0
October 2002								
1-7	30.4	23.3	26.9	89	37.7	7.0	5.6	365.3
8-14	29.8	23.0	26.4	90	89.0	2.6	10.4	258.0
15-21	30.3	22.0	26.2	88	18.6	4.7	7.3	328.7
22-28	30.9	22.6	26.8	92	17.8	4.6	3.7	290.4
29-4	28.4	21.7	25.1	80	106.4	5.4	3.9	317.7
November 2002								
5-11	29.6	22.6	26.1	94	42.6	3.6	3.8	296.1
12-18	30.0	21.7	25.9	91	13.5	5.5	4.0	348.4
19-25	29.0	21.1	25.1	89	4.0	4.7	4.7	336.6

Table 5. Temperature regimes for various treatments in Phytotron

Time (hrs)	Treatment temperatures (^{o}C)													
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}
06 – 10	34	33	32	32	32	31	30	30	30	28	28	28	27	27
10 – 16	36	35	34	34	34	33	33	32	31	30	30	29	29	28
16 – 20	32	31	30	30	30	29	28	28	28	26	26	26	25	25
20 – 26	24	23	23	22	21	21	20	20	20	20	19	19	18	18
Max (^{o}C)	36	35	34	34	34	33	33	32	31	30	30	30	29	28
Min (^{o}C)	24	23	23	22	21	21	20	20	20	20	19	19	18	18
Daily mean (weighted average)	30.0	29.0	28.5	28	27.5	27.0	26.5	26.0	25.5	25.0	24.5	24.0	23.5	23.0

Day length: 10.00 hrs/day (08:00 – 18:00 hrs), RH: 60 – 80%,

Light: 400 – 800 µ moles m^{-2} s^{-1}

CHAPTER IV

EXPERIMENTAL RESULTS

An investigation was carried out to study the critical sterility temperature and the critical stage of thermosensitivity of the selected thermosensitive line TS 29 using Phytotron, in the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore. The physiological, biochemical and molecular changes during fertility / sterility transformation were also studied to understand the underlying mechanism responsible for male sterility in TS 29 line and to reveal the possibility of manipulating sterility or fertility using chemicals and growth regulators. Field experiments were conducted during Rabi, 2001-2002 and Kharif, 2002 for induction of male sterility / fertility at Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore. The crop was sprayed with appropriate chemicals and growth regulators at stage III and stage V of panicle development. The biochemical aspects were also studied after the induction treatments. The selective sterility of the chemical on the pollen grains leaving the female organs fertile was studied under fluorescence microscope, and the morphological and anatomical 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. THERMOSENSITIVITY AND ATTENDANT CHANGES DURING REPRODUCTIVE PHASE OF TS 29 IN COMPARISON WITH CO 46

Obviously, rice plant is influenced by changes in temperature at its growing environment. Among the phenophases, several reports have indicated (Table 2 and 3) that the reproductive phase, particularly the developing reproductive organs seem to be influenced by the changes in temperature. In order to precisely evaluate the effect of different temperature regimes on induction of sterility and to understand the biochemical background for such changes, certain

experiments were conducted involving the thermosensitive rice line TS 29. In addition, the popular cultivar CO 46, a relatively thermoinsensitive one, was also involved in the studies for comparison (Plate 1).

4.1.1. Critical sterility temperature (CST) of TS 29

Experiment was carried out to fix the critical sterility temperature of TS 29 during the reproductive stage. Table 12 shows the pollen sterility percentages of TS 29 at different daily mean temperatures. The pollen sterility percentage increased steeply as the daily mean temperature increased and in TS 29, the pollens were 100 per cent sterile at 27.5^{o}C (34.0 / 21^{o}C). The pollen sterility percentage for the daily mean temperature of 27^{o}C (33^{o}C maximum and 21^{o}C minimum temperatures) was 97.5 which is on par with that of 27.5^{o}C (100%). For every drop of 0.5^{o}C mean temperature below 27^{o}C, there was steep decrease in pollen sterility, and below 24^{o}C there were negligible sterility values. The results suggested that for effecting 100 per cent pollen sterility, the CST of TS 29 was 27.5^{o}C. At the daily mean temperature of 29.0^{o}C and above, the anthers were found completely pollen free.

4.1.2. Critical stage of thermosensitivity

TS 29 was exposed to CST (27.5^{o}C) during different stages of panicle development to identify the exact critical stage of thermosensitivity for fertility alterations. The line exhibited 100 per cent pollen sterility when exposed to CST during 0 to 30 days before heading (DBH), 5 to 25 DBH and 10 to 25 DBH (Table 13). Exposures during 0 to 20 DBH, 5 to 30 DBH, 10 to 30 DBH and 10 to 20 DBH recorded pollen sterility values ranging from 92.5 to 95.5 per cent. CST treatment during the other durations of panicle development stage resulted in pollen sterility of 66 per cent and below. There were significant variations among the treatments. The results indicated that the critical stage of thermosensitivity for fertility alteration in TS 29 was 10 to 25 days before heading, as it was the minimum period of exposure to CST to get complete pollen sterility.

4.1.3. Physiological and biochemical changes under pollen fertile and sterile conditions

Under Phytotron conditions, CST treatment was given during 10 – 25 days before heading which coincides the period between stage III and stage VII of panicle development. Simultaneously, the plants were also maintained at Glass house condition, where the mean temperature during the corresponding period of panicle development ranged from 22^{o}C to 24.25^{o}C, which resulted in fertile pollens (Plate 2). CO 46 also was tested along with TS 29 under both these conditions to understand the relative performance of TS 29 as compared to CO 46. Plant samples were taken immediately after stage VII of panicle development for physiological and biochemical analysis.

4.1.3.1. IAA and ABA contents in leaves and emerging panicles

Data on IAA and ABA contents estimated in leaves and young panicles of TS 29 and CO 46, raised under both of Glass house and Phytotron conditions, are furnished in Table 14. All the values exhibited significant differences among the treatments. IAA contents were high in both leaves and young panicles of TS 29 plants raised under Glass house condition which produced fertile pollens. In Phytotron raised plants of TS 29 that resulted in sterile pollens, the decrease in IAA content was to the tune of 16.84 per cent in leaves, whereas, it was only 9.1 per cent in young panicles. But in both Glass house and Phytotron raised plants of CO 46, the variety being relatively insensitive to temperature changes, produced only fertile pollens. The differences in IAA contents in leaves and in young panicles of CO 46 were negligible only with a decrease of 2.33 per cent in leaves and 0.2 per cent in panicles. However, comparatively CO 46 recorded higher IAA contents than TS 29 in both the situations.

Unlike the narrow differences in IAA contents, ABA content decreased very steeply in both leaves and young panicles when TS 29 happened to produce sterile pollens due to CST

treatment in Phytotron. Under Glass house condition, the values of ABA contents in leaves and young panicles were 0.584 and 0.401 respectively. But, the corresponding values under Phytotron conditions (producing sterile pollens) were 0.157 and 0.184 respectively. The results suggested that there was 73.1 per cent reduction in ABA content in leaves and 54.1 per cent in young panicles due to the treatment of critical sterility temperature to TS 29 under Phytotron condition during panicle development stage. On the other hand, there were minimum changes in ABA contents both in leaves and young panicles of CO 46 between the plants raised in both the environments. But in leaves of CO 46, the ABA values were about three to six times higher than that of TS 29. However, young panicles of TS 29, producing fertile pollens, recorded substantially higher contents of ABA (0.401) as compared to CO 46 (0.245). Under Phytotron condition, CO 46 produced slightly increased content of ABA as compared to that of Glass house plants, however both values being on par statistically.

4.1.3.2. Ethylene release rate from emerging panicles (µ moles min^{-1})

Ethylene release rates (ERR) from the emerging panicles of TS 29 and CO 46 raised under Glass house and Phytotron conditions, were recorded and furnished in Table 15. By subjecting TS 29 to critical sterility temperature under Phytotron, the ethylene release from the panicles was steeply increased to the tune of 234 per cent as compared to that raised under Glass house condition which produced fertile pollens. The plants producing sterile pollens recorded ethylene concentration value of 5.621, while the plants raised under Glass house condition recorded only 2.403. In CO 46, there was no difference in ethylene concentration of emerging panicles of plants raised under Glass house (1.999) and Phytotron (2.011). The area and height of ethylene peak also followed the same trend.

4.1.3.3. Gas exchange parameters in leaves

Gas exchange parameters, namely photosynthetic rate (P_n), transpiration rate (E) and diffusive resistance (r_s) were recorded in the leaves of TS 29 and CO46,raised under Glass house

(without CST treatment) and under Phytotron (with CST treatment during panicle development) conditions. The values of these gas exchange parameters recorded at stage VII of panicle development are furnished in Table 16.

Photosynthetic rate (P_n) (μ mol CO_2 m^{-2} s^{-1})

The photosynthetic rate values decreased steeply in TS 29 from 20.5 in plants raised under Glass house condition to 13.1 in plants raised under Phytotron, which received critical sterility temperature treatment during panicle development resulting in sterile pollens. Though CO 46 was also subjected to similar treatments, the photosynthetic rate value was 22.5 under Glass house and 20.16 under Phytotron. The decrease in photosynthetic rate was not only minimum due to CST treatment in Phytotron, but also has not resulted in sterile pollens in the case of CO 46. The results indicated that a steep decrease of 36.1 per cent P_n due to CST treatment seemed to be closely associated with rendering the pollens sterile in TS 29, whereas, a narrow decrease of 10.4 per cent P_n in CO 46 due to CST treatment seemed to have spared the pollens of this variety with full fertility.

Transpiration rate (E) (m mol H_2O m^{-2} s^{-1})

Unlike P_n, the changes in E values between Glass house and Phytotron raised plants of TS 29 and CO 46 were narrow. The rates were 7.5 and 7.0 in Glass house and Phytotron raised TS 29, plants respectively, and 7.7 and 7.l5 in CO 46 respectively. Only in TS 29, the difference was statistically significant. It appeared that the E reduced moderately in TS 29 and only negligibly in CO 46 by giving CST treatment during panicle development under Phytotron as compared to the plants raised under Glass house condition with relatively lower atmospheric temperature of about 24^{o}C.

Diffusive resistance (r_s) (s cm^{-1})

By giving CST treatment during panicle development to TS 29 under Phytotron condition, the plants gained 14.3 per cent increased diffusive resistance over the plants raised

under Glass house without CST. The r_s values were 6.4 and 5.6 respectively. But, CO 46 plants which have undergone similar treatments failed to exhibit significant differences in this parameter. The increase in r_s due to CST treatment would have been the reason for reduced transpiration rate in plants receiving CST treatment.

4.1.3.4. Carbohydrate fractions in leaves

Reducing sugars, total sugars, starch content and total carbohydrates in leaves of both TS 29 and CO 46, raised under Glass house and Phytotron conditions are furnished in Table 17. By CST treatment, TS 29 produced sterile pollens under Phytotron and fertile pollens without CST under Glass house. Under both the conditions, CO 46 produced fertile pollens only. The changes in carbohydrate fractions in TS 29 and CO 46, subjected to the two treatments, were recorded for getting probable relationships of these fractions with fertility / sterility inductions.

Reducing sugars (mg g^{-1})

TS 29, under Glass house condition that produced fertile pollens, recorded reducing sugars content of 7.52 mg g^{-1}, while Phytotron raised plants that produced sterile pollen had only 5.61 mg g^{-1}. The reduction in reducing sugar due to CST treatment was about 25.4 per cent, which might be one of the causes for pollen sterility. Curiously, there was no significant reduction in reducing sugars content of CO 46 by CST treatment.

Total sugars (mg g^{-1})

The trend in total sugars was almost similar to that of reducing sugars, but the difference in total sugars contents between Glass house and Phytotron raised TS 29 plants was still more wide with 30.3 per cent reduction in CST treated Phytotron plants of TS 29, as compared to Glass house plants without CST. In total sugars also, there was insignificant and negligible decrease in CO 46 subjected to CST.

Starch content (mg g^{-1})

Compared to Glass house raised TS 29 plants which recorded starch content of 37.14 mg g^{-1}, the TS 29 plants raised under Phytotron that produced sterile pollens due to CST treatment recorded significantly higher starch content value of 51.26 with 38 per cent increase over that of Glass house plants. CO 46 failed to exhibit any difference in starch contents between both Glass house and Phytotron raised plants. There was no sterility also in CO 46 due to CST treatment.

Total carbohydrates (mg g^{-1})

Total carbohydrates followed the trend of total sugars by recording reduced content of 65.43 mg g^{-1} in TS 29 receiving CST treatment, while the same line raised under Glass house without CST recorded 77.91 mg g^{-1}. The results suggested that the reduction in total carbohydrates also might be a cause for producing sterile pollens due to CST. But, the same CST treatment failed to produce sterile pollens in CO 46 and so there was almost similar values of total carbohydrates in both Glass house and Phytotron raised plants of CO 46 indicating the stability of the cultivar to temperature variations.

4.1.3.5. Hill reaction rate and nucleic acid contents in leaves

Hill reaction rate (µ moles DCIP reduced mg^{-1} chlorophyll hr^{-1})

A high hill reaction rate value of 39.68 was recorded in TS 29 producing fertile pollens under Glass house condition, while about 31 per cent reduced rate of 27.41 hill reaction value was recorded in TS 29, subjected to CST treatment under Phytotron that produced only sterile pollens (Table 18). Comparatively, CO 46 produced almost uniform hill reaction rates both under Glass house and Phytotron conditions, and so, CO 46 produced fertile pollens under both the situations.

Nucleic acid contents (µg g^{-1})

Both RNA and DNA contents were high when fertile pollens were produced (TS 29 under Glass house, CO 46 under both Glass house and Phytotron conditions). In TS 29 plants subjected to CST treatment during panicle development stage under Phytotron, both RNA and DNA contents were reduced by 30.55 per cent and 37.5 per cent respectively as compared to that of plants producing fertile pollens under Glass house condition (Table 18). The differences were significant only in TS 29 involved treatments, whereas in CO 46, only negligible differences existed between the treatments resulting in fertile pollens under both the situations.

4.1.3.6. Certain biochemical constituents in leaves

Soluble protein, proline, total phenols, total free amino acids and ascorbic acid contents were estimated in the leaves of TS 29 and CO 46, raised under Glass house and Phytotron conditions, at stage VII of panicle development, and the data are furnished in Table 19. TS 29 alone produced sterile pollens when subjected to CST treatment in Phytotron, while CO 46 produced fertile pollens under similar situation. To elicit any possible relationships of the above biochemical constituents with the induction of pollen sterility by CST treatment, the contents of these were estimated.

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

Among the two cultivars, CO 46 recorded the maximum soluble protein content of 8.21 mg g^{-1} under Glass house condition with only a negligible decrease when the plants were subjected to CST treatment (8.17 mg g^{-1}). But in TS 29 line, the soluble protein content was 7.66 under Glass house condition and the content was drastically reduced (4.62 mg g^{-1}) when the line was subjected to CST treatment resulting in sterile pollen. By effecting about 39.3 per cent reduction in soluble protein content, apparently the pollens might have been rendered sterile by CST treatment.

Proline content (mg g^{-1})

By increasing the temperature to the critical sterile temperature of 27.5^{o}C over the ambient temperature of about 24^{o}C under Glass house condition, proline content was almost doubled in TS 29, while the increase in proline content was about 38 per cent in CO 46. It is apparent that, in a thermosensitive line like TS 29, the production of proline is of high order culminating in induction of pollen sterility.

Total phenols (mg g^{-1})

Total phenols also showed wide difference between Glass house raised TS 29 and Phytotron raised TS 29 receiving CST treatment. About 42 per cent increase in phenol content was observed due to CST treatment in TS 29. But the same treatment failed to reveal any significant difference in CO 46. It seems that, in consequence to CST treatment, total phenols increased substantially in thermosensitive TS 29 leading to production of sterile pollens.

Total free amino acids (mg g^{-1})

While producing fertile pollens in TS 29 or CO 46, the total free amino acid contents ranged between 2.77 and 2.99. As a result of CST treatment, when TS 29 plants were influenced to produce sterile pollens, about 55 per cent reduction in total free amino acids content was observed. This steep decrease in total free amino acid contents might be one of the reasons for causing pollen sterility in TS 29 due to CST treatment. CO 46, being a thermoinsensitive cultivar, failed to reveal any significant change in total free amino acid content after the CST treatment.

Ascorbic acid content (mg g^{-1})

The ascorbic acid contents of CO 46 were found higher (70.96 and 69.45) than that of TS 29, which recorded 56.83 mg g^{-1} under Glass house and 36.64 mg g^{-1} under Phytotron after receiving CST treatment. About 35.5 per cent decrease in ascorbic acid content in TS 29 might

have contributed to the induction of pollen sterility consequent to CST treatment. Only a minimum difference in ascorbic acid content were seen in CO 46 between the two treatments.

4.1.3.7. Enzyme activities in leaves

Activities of certain important enzymes namely, IAA oxidase, nitrate reductase, ascorbic acid oxidase and glutathione dehydrogenase were analysed in leaves of TS 29 and CO 46 after giving CST during panicle development under Phytotron condition, and simultaneously raising the two cultivars under Glass house condition without subjecting them to CST. The data are furnished in Table 20.

IAA oxidase (µ moles of unoxidised auxin g^{-1} hr^{-1})

IAA oxidase activity expressed in terms of µ moles of unoxidised auxin g^{-1} hr^{-1} of TS 29 line under Glass house and Phytotron conditions in comparison with normal variety, CO 46 during stage VII of panicle development is given in Table 20. The enzyme activity increased considerably under sterile condition. The unoxidised auxin accumulated under fertile and sterile conditions were 15.62 µ moles of unoxidised auxin g^{-1} hr^{-1} and 9.31 µ moles of unoxidised auxin g^{-1} hr^{-1} respectively with a reduction of 40.4 per cent. The unoxidised auxin in CO 46 under Glass house and Phytotron were 15.33 and 14.91 values respectively. Results indicated that there was increased activity of IAA oxidase under sterile condition depleting the auxin content.

Nitrate reductase activity (NR activity) (µ moles NO_2 g^{-1} hr^{-1})

CO 46 recorded very high activities of 0.645 and 0.640 values under Glass house and Phytotron conditions respectively, with minimum, but significant differences. Conversely, TS 29 under Glass house condition recorded 'NR' activity value of 0.582, while that under Phytotron recorded 0.341 value with a considerable decrease in the activity of about 41.4 per cent. It is

apparent that CST treatment has seriously hampered the 'NR' activity in the thermosensitive TS 29 line as compared to the thermoinsensitive CO 46.

Ascorbic acid oxidase activity (AAO activity) (Enzyme units g^{-1} min^{-1})

About 253 per cent increased AAO activity was recorded when TS 29 was subjected to CST treatment under Phytotron compared to non-CST treatment under Glass house. TS 29 recorded AAO activity values of 3.33 and 8.42 under Glass house and Phytotron conditions respectively, whereas the corresponding values for CO 46 were 2.12 and 2.99. Though CO 46 also revealed significantly increased activity under Phytotron, the increase was not so enormous, as seen in TS 29. The results clearly expressed that the increased AAO activity could be harmful to pollen fertility.

Glutathione dehydrogenase activity (GDH activity) (nmoles of NADH oxidised mg^{-1} protein min^{-1})

Very high GDH activities of 2.76 and 2.51 were recorded in TS 29 and CO 46 under Glass house condition respectively. But their activities under Phytotron after receiving CST treatment were 0.92 and 1.99 respectively. The reduction in the activity was as low as 66.7 per cent in TS 29, whereas, the reduction was only 20.7 per cent in CO 46. The steep reduction in GDH activity as a result of CST treatment in TS 29 has to be taken note of for sterility induction in this line.

4.1.3.8. Activities of active oxygen species scavenging enzymes in leaves

With a view to understand the effect of giving critical sterility treatment to both thermosensitive TS 29 and thermoinsensitive CO 46, and to compare the changes taking place in these cultivars under sub-optimal critical temperature, in terms of important enzymes involved in scavenging active oxygen species namely catalase, peroxidase and superoxide dismutase, leaf samples from these two cultivars were analysed at stage VII of panicle development after the

respective treatments were given. The data generated from these analysis are furnished in Table 21.

Catalase activity (Enzyme units × 10^4 g^{-1} min^{-1})

CO 46 recorded very high catalase activity values of 3.92 and 3.81 under Glass house and Phytotron conditions respectively. But in thermosensitive TS 29, a very wide variation in catalase activity was observed with Glass house plants recording the value of 2.98, while the Phytotron raised plants that received CST treatment recording a value of 1.45. By CST treatment, catalase activity was found to decline to the extent of 51.3 per cent as compared to the Glass house plants. Though significant differences were recorded in CO 46, the difference between Glass house and Phytotron plants was very narrow (2.8 per cent).

Peroxidase activity (Enzyme units l^{-1} hr^{-1})

By providing CST treatment, peroxidase activity was found decreased in both TS 29 and CO 46, but the range was very wide in TS 29 with values 175 in Glass house treatment and 129 in Phytotron treatment. The decrease in activity due to CST treatment was as high as 26.3 per cent in TS 29. But in CO 46, the decrease was only 6.5 per cent, even though the activity in CO 46 was comparatively lower than TS 29, especially under Glass house condition.

Superoxide dismutase (SOD) (Enzyme mg^{-1} protein min^{-1})

The values of superoxide dismutase exhibited almost similar trend as that of catalase activity. CO 46 performed with higher SOD activity, both under Glass house and Phytotron conditions, the values being 9.16 and 8.77. But, the activity was found to seriously decline as TS 29 was given critical sterility temperature when the SOD value was only 4.11. Whereas, under Glass house condition, it recorded a value of 8.62. The decrease due to CST treatment was found to be about 52.3 per cent. It appears that for production of fertile pollens, high SOD activity is essential.

4.1.3.9. Nutrient status of leaves

Leaf samples were collected at stage VII of panicle development in both TS 29 and CO 46 cultivars. Both the cultivars were simultaneously raised under Glass house and Phytotron conditions (subjecting the plants to CST treatment during panicle development). Important nutrients like N, K, Ca, Cu and B were analysed from the leaf samples and the results are presented in Table 22.

Nitrogen (N) content (%)

Both TS 29 and CO 46 recorded almost similar values (2.99 and 3.01 per cent respectively) when raised under Glass house condition. But, the 'N' content was only 2.49 per cent in TS 29, while CO 46 recorded 2.94 per cent under Phytotron condition. Compared to Glass house raised plants, the reduction in total nitrogen percentage in TS 29 under Phytotron was about 17 per cent, whereas, it was only about 2.3 per cent in CO 46.

Potassium (K) content (%)

Similar to nitrogen content, potassium content in leaves of TS 29 and CO 46 were on par with each other when raised under Glass house condition. But under Phytotron condition, the leaf 'K' content was 1.03 in TS 29, whereas, it was 1.4 in CO 46. By giving CST treatment to TS 29, a substantial reduction to the extent of 32.2 per cent was noticed in 'K' content, whereas, the reduction was only 6 per cent in CO 46.

Calcium (Ca) content (µg g^{-1})

Calcium also behaved almost similar to 'N' and 'K' contents. Though significant differences existed, the 'Ca' contents of CO 46 under Glass house and Phytotron conditions, and that of TS 29 under Glass house condition revealed almost similar values. But the content was

reduced by 23.6 per cent when TS 29 was subjected to CST treatment under Phytotron condition as compared to that under Glass house condition.

Copper (Cu) content (µg g^{-1})

A reduction of 22.6 per cent in 'Cu' content was observed in TS 29 when CST treatment was given as against the values recorded in the line, raised under Glass house condition. But, CO 46 was found to have comparatively higher 'Cu' contents than TS 29. Further, the reduction in 'Cu' content due to CST treatment in CO 46 was very narrow (8.3 per cent).

Boron (B) content (µg g^{-1})

Among the several nutrients analysed in the present study, 'B' contents showed wide variations between TS 29 plants raised under Glass house and Phytotron conditions (39.0 and 22.0 µg g^{-1}). The reduction in content due to CST treatment was as high as 43.6 per cent. On the contrary, the 'B' contents of both Glass house and Phytotron raised CO 46 plants showed insignificant difference.

4.2. RELATIVE BIOCHEMICAL CHANGES IN TS 29 PRODUCING FERTILE AND STERILE POLLENS

4.2.1. Free amino acids

The list and quantity of individual amino acids, present in sterile and fertile TS 29, are presented in Table 23. The results showed that the essential amino acids namely lysine (basic amino acid), tyrosine (aromatic amino acid) and isoleucine (aliphatic amino acid) were absent under sterile condition, whereas, the other amino acids namely leucine, cysteine, methionine and histidine were present in decreased amounts (0.009 mg 100 g^{-1}, 0.132 mg 100 g^{-1}, 0.120 mg 100 g^{-1} and 0.098 mg 100 g^{-1} respectively) compared to the fertile TS 29 line, which recorded higher contents. The amino acids isoleucine and tyrosine were found in higher quantities of 0.290 mg 100 g^{-1} and 0.277 mg 100 g^{-1} respectively, whereas, leucine and

methionine contents were 0.139 mg 100 g^{-1} and 0.137 mg 100 g^{-1} respectively in the fertile plants of TS 29. Absence of essential amino acids and reduced presence of certain other amino acids appeared to negate the pollen fertility in a thermosensitive line.

4.2.2. Individual phenols

The list and quantity of individual phenols under sterile and fertile phases of TS 29 line are given in the Table 24. The HPLC profile showed that none of the phenols were detected under fertile phase, while salicylic acid, catechol and pyrocatechol alone were detected under sterile condition (Plate 3). The estimated quantities of these phenols under sterile phase were salicylic acid 5.34 mg g^{-1} followed by pyrocatechol 3.61 mg g^{-1} and catechol 1.73 mg g^{-1}. The study indicated the deleterious effect of phenols on pollen fertility.

4.2.3. Polyamines

Distinct differences were observed in polyamine contents of TS 29 line under sterile and fertile conditions (Table 25). The fertile plant leaves showed higher amounts of spermidine (2.13 µ mol g^{-1}) followed by cadavarine (0.39 µ mol g^{-1}), while the polyamine putrescine was not detected. In the sterile leaves, putrescine (3.84) was found to be the major polyamine followed by cadavarine (0.33). Spermidine was not detected in sterile leaves. The amount of polyamines, present in the panicles was less as compared to the leaves. In the fertile panicle, only spermidine (0.05 µ mol g^{-1}) was detected, while in the sterile panicle, putrescine (0.03 µ mol g^{-1}) was detected (Plate 4). The results suggested that the putrescine was the major polyamine under sterility and spermidine was predominant under fertile conditions.

4.3. EFFECT OF CHEMICALS AND GROWTH REGULATORS ON STERILITY INDUCTION IN TS 29

In the present study, the critical temperature for effecting 100 per cent sterility in TS 29 has been fixed at 27.5^{o}C (Table 12). However, under field conditions, the critical sterility temperature

may be still higher due to other influences like humidity and wind velocity. Under Coimbatore conditions, complete sterility in TS 29 under field condition was observed only when the panicle development stage coincides with high temperature regimes with mean temperature range of 28.25 to 29^{o}C during April, May and June (Latha, 2001). During other periods, only partial sterility was recorded, which hampered hybridization programme involving TS 29. To overcome the difficulty experienced by breeders in this context, certain chemicals and growth regulators were sprayed during stage III and stage V of panicle development phase, with a view to get increased sterility. The sowings of the field crops were so adjusted that the mean temperatures during panicle development stage did not reach the CST. In the first season, sowing was taken up on 14.12.2001, and the critical stage of thermosensitivity coincided the period between 6.2.2002 and 21.2.2002, during which period, the mean temperature ranged between 25.4^{o}C and 26.2^{o}C. The sowing in the second season was taken up on 12.7.2002, and the critical stage of thermosensitivity for this crop was between 6.9.2002 and 21.9.2002, during which period, the mean temperature ranged between 25.9^{o}C and 26.9^{o}C. Salicylic acid (800 and 1000 ppm), ethrel (800 ppm) and maleic hydrazide (2000 ppm) were sprayed during IIIrd and Vth stages of panicle development. Untreated control treatment was included for comparison. Plant samples were collected five days after first spray and five days after second spray and also during flowering for analysis of various biochemical constituents.

4.3.1. Effect of chemicals and growth regulators on pollen sterility

During season I, pollen sterility percentage ranged from 57.22 in control to 99.95 in 1000 ppm salicylic acid. But in season II, only 17.04 per cent pollen sterility was recorded in control, and the maximum sterility of 98.78 per cent was also recorded in salicylic acid (1000 ppm) treatment. In both the seasons, the differences among treatments were statistically significant. But, the values recorded in salicylic acid at 800 and 1000 ppm treatments, in both the seasons, were on par with each other and hence, it can be construed that 800 ppm concentration of salicylic acid is optimum for obtaining almost complete pollen sterility. Other than salicylic acid, ethrel at 800 ppm and maleic

hydrazide at 2000 ppm concentration effected moderately higher pollen sterility of about 20 per cent, but far below that of salicylic acid treatment (Table 26).

4.3.2. Effect of chemicals and growth regulators on percentage of crossed seed set

Crossed seed set percentages were recorded after employing rope pulling method and clipping method in both the seasons. The data are furnished in Table 27. All the values, under both the methods employed in two seasons, revealed significant variations among treatments. Seed set in control plants was not taken into account, as only partial sterility was observed. The data clearly revealed that by spraying salicylic acid (800 ppm) concentration during panicle development stage, maximum seed set percentages were recorded in every season under both the methods. But, the values were almost on par with that of salicylic acid at 1000 ppm. Among the other two treatments, maleic hydrazide at 2000 ppm recorded comparatively increased crossed seed set percentage over that of ethrel at 800 ppm. Among the two methods adopted, clipping method appeared to be far better than rope pulling method in enhancing seed set percentage.

4.3.3. Effect of chemicals and growth regulators on certain biochemical constituents

4.3.3.1. Starch content (mg g^{-1})

Data on starch content, analysed in leaves of TS 29 during three different stages after panicle initiation in two seasons, revealed significant differences among the treatments (Table 28). In samples taken after first chemical spray, salicylic acid at 1000 ppm showed maximum starch content in the first season, whereas, plants that received no spray and also plants sprayed with maleic hydrazide (2000 ppm) recorded very high starch contents in the second season crop. But, subsequent leaf samples taken during fifth stage of panicle development and at flowering showed increased starch content in 1000 ppm salicylic acid spray. Salicylic acid 800 ppm also performed better apart from recording highest starch content during flowering stage of second season. The data indicated that salicylic acid spray, either at 800 or 1000 ppm concentration, could enhance starch content in leaves of TS 29.

4.3.3.2. Total carbohydrates (mg g^{-1})

Total carbohydrate contents of leaves of TS 29 were analysed in samples taken five days after first spray, five days after second spray and during flowering. Data recorded in two seasons during the above stages showed clear differences among the treatments (Table 29). In all the stages sampled, plant samples from the control treatment recorded highest total carbohydrates content in all the sampling intervals of both the seasons. Among the chemical sprayings, maleic hydrazide (2000 ppm) followed by ethrel (800 ppm) treatments performed better in terms of carbohydrate contents. Salicylic acid treatments portrayed low carbohydrate contents in all the sampling intervals.

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

Soluble protein contents of TS 29 were analysed in leaf samples collected on fifth day after first spray (Stage III), fifth day after second spray (Stage V) and during flowering. The data are furnished in Table 30, which revealed significant differences. Maximum soluble protein contents were recorded in all the samples collected from control plants. Salicylic acid ,either at 800 ppm or 1000 ppm concentrations, recorded decreased soluble protein contents in all the sampling intervals and seasons as compared to other treatments. However, the data failed to reveal distinct differences among treatments. In general, soluble protein content values were lower during early panicle development stage and thereafter, there was a large increase in soluble protein content.

4.3.3.4. Proline content (mg g^{-1})

Salicylic acid spray, either at 1000 or at 800 ppm, during third and fifth stages of panicle development of TS 29, was found to increase proline content of leaves significantly over other chemical treatments or untreated control (Table 31). In both the seasons, salicylic acid sprays produced distinctly superior values. Both ethrel and maleic hydrazide sprays performed almost uniformly with comparatively lower values of proline content than salicylic acid treatments, but far

higher than the values of control treatment. The data indicated that the plants devoid of any of the given spray treatments might record only minimum proline content values. Among the stages, during early panicle development the proline contents were low with a range of 0.83 to 1.22 mg g^{-1}. But, during late panicle development and flowering stages, about three to four fold increase in proline contents was noticed in many of the treatments with a range of 1.42 to 5.35 mg g^{-1}.

4.3.3.5. Total phenols (mg g^{-1})

Salicylic acid spray at 800 ppm concentration was found to effect definite increase in total phenols in many of the stages of sampling in both the seasons followed by salicylic acid (1000 ppm) (Table 32). Both ethrel and maleic hydrazide sprays were not as effective as salicylic acid in enhancing the phenol content. In untreated control treatment, the leaves of TS 29 recorded comparatively far lower content of total phenols than other treatments. The phenol content also increased with the advancement of plant duration after panicle initiation and high phenol contents were recorded during flowering stage as compared to other stages. Salicylic acid spray appeared to trigger increased production of phenols, as evident from the present study.

4.3.3.6. Individual phenols (mg g^{-1})

Individual phenols were analysed from leaf samples collected after giving second chemical spray during panicle development in the second season crop (Table 33). In control treatment, only syringic acid was estimated to the value of 1.601 mg g^{-1} ,and other phenols were not detected. By spraying salicylic acid 800 ppm and 1000 ppm concentrations, the salicylic acid levels in leaves were to the tune of 6.312 and 7.728 mg g^{-1} respectively. Salicylic acid 800 ppm spray also resulted in the production of 5.553 mg g^{-1} benzoic acid and 1.104 mg g^{-1} catechol. But, salicylic acid 1000 ppm appeared to favour the production of ferulic acid (1.057 mg g^{-1}) and pyrocatechol (3.655 mg g^{-1}), apart from producing benzoic acid and catechol (3.751 and 1.673 mg g^{-1} respectively). Ethrel spray resulted in the production of salicylic acid (1.053 mg g^{-1}), ferulic acid (1.112 mg g^{-1}) and a meagre amount of catechol (0.052 mg g^{-1}). Maleic hydrazide spray favoured only ferulic acid

(2.199 mg g^{-1}) and catechol (2.674 mg g^{-1}), but the contents of these two phenols were far lower in other chemical treatments.

4.3.3.7. Total free amino acids (mg g^{-1})

Total free amino acid values in the leaves of TS 29 ranged from 1.54 to 2.73 in all the three stages of sampling taken during both the seasons. However, significant differences were observed among the treatments (Table 34). In the first season samples, control plants recorded high content of free amino acid as compared to all the other treatments. But during second season, salicylic acid 800 ppm recorded comparatively increased content of total free amino acids in all the sampling stages. Ethrel and maleic hydrazide treatments recorded almost uniform values with negligible differences.

4.3.3.8. Ethylene release rate from panicles (µ moles min^{-1})

The rate of ethylene release from emerging panicles of TS 29 was estimated in different treatments during second crop season (Table 35). The ethylene concentration was very high in salicylic acid 1000 ppm treatment (5.989). This was followed by ethrel 800 ppm (4.808) and salicylic acid 800 ppm (4.765) and both values were on par with each other. The plants which received no chemical treatment (control) were found to release very low quantity of ethylene during panicle emergence (1.906).

4.3.4. Effect of chemicals and growth regulators on activities of certain enzymes

4.3.4.1. IAA oxidase activity (µ moles of unoxidised auxin g^{-1} hr^{-1})

Though significant differences were observed among the treatments with regard to IAA oxidase activity in leaf samples taken during the two stages of panicle development, the range of differences were very narrow in both the seasons (Table 36). During first sampling stage of panicle development, IAA oxidase values ranged between 15.77 (Ethrel 800 ppm) and 15.25 (Control). In the

samples taken at second stage of panicle development, the activity ranged between 15.02 and 16.63. But, clear and wide differences were exhibited by the treatments only during flowering, when very low IAA oxidase activity values of 17.23 and 16.02 were recorded in control treatment during first and second season respectively. All other chemical treatments recorded far higher activities than the control treatment. Among the chemicals and growth regulators, maleic hydrazide (2000 ppm) revealed slightly decreased activity over other chemical treatments.

4.3.4.2. Nitrate reductase activity (µ moles NO_2 g^{-1} hr^{-1})

Data on nitrate reductase activities in leaves of TS 29, collected during two stages of panicle development and during flowering, revealed significant differences among the treatments, with control treatment recording increased nitrate reductase activity over the chemical treatments (Table 37). The differences in activities widened with the advancement of age and at flowering, the activity in control plants was far ahead of other treatments with values of 0.424 and 0.625 during first and second seasons respectively, while all the other treatments recorded values below 0.4. The pooled mean of two seasons values indicated that the activity was low in maleic hydrazide treatment. The data indicated that the chemicals and growth regulators, used in this study, were not conducive to enhance the activity of nitrate reductase in TS 29. Being an important metabolic enzyme, lowering the activity of nitrate reductase at the panicle development stage by the chemicals might have harmfully affected the pollen fertility in TS 29.

4.3.4.3. Ascorbic acid oxidase activity (Enzyme units g^{-1} min^{-1})

Ascorbic acid oxidase activity values, obtained from the analysis of leaf samples taken during two stages of panicle development and during flowering of TS 29, revealed the supremacy of salicylic acid treatments in keeping the ascorbic acid oxidase activity at increased levels (Table 38). Either 1000 ppm or 800 ppm concentrations of salicylic acid showed increased enzyme activity among the treatments in all the sampling stages of both the seasons. Very low activity was found in control plants while, the plants that received either ethrel or maleic hydrazide sprays also performed better with moderately higher activity than the control plants.

4.3.4.4. Catalase activity (Enzyme units x 10^4 g^{-1} min^{-1})

Catalase activity was continuously high in control in plants during all the three stages of sampling of both the seasons (Table 39). The differences were statistically significant. Among the chemical treatments, both ethrel and maleic hydrazide recorded fairly increased catalase activity over salicylic acid treatments. Pooled mean of both the seasons values, recorded during panicle development stages and at flowering, exhibited consistently low activity in salicylic acid 800 ppm treatment followed by salicylic acid 1000 ppm. It is apparent from the data that catalase activity was maintained at low level, when salicylic acid either 800 or 1000 ppm concentration was sprayed on TS 29.

4.3.4.5. Peroxidase activity (Enzyme units l^{-1} hr^{-1})

Similar to catalase activity, peroxidase activity also was found high in untreated control plants of TS 29 during all the three sampling intervals of both the seasons (Table 40). The treatmental differences were statistically significant and the differences widened with advancement of age upto flowering. The values of peroxidase activity during flowering in control plants ranged between 182.6 to 184.4 units. While in salicylic acid treatments, the activity ranged between 142.3 and 145.4. Ethrel and maleic hydrazide showed moderate activities, but distinctly higher than salicylic acid treatments and far lower than control treatment (154.5 to 162.5).

4.3.4.6. Superoxide dismutase (Enzyme units mg^{-1} protein hr^{-1})

Superoxide dismutase activity remained almost unchanged in control plants during the course of the chosen developmental stages in both the seasons (Table 41). The values ranged between 8.25 to 8.50. But by spraying salicylic acid, superoxide dismutase activity values declined from about 6.3 to about 5.0. Ethrel and maleic hydrazide treatments revealed an intermediate trend of activity during the three stages of sampling. The data clearly exhibited that

the chemicals and growth regulators, used in the study, were able to reduce superoxide dismutase activity, and among them, salicylic acid spray expressed distinct reduction in the activity of this enzyme.

4.3.5. Effect of chemicals and growth regulators on certain nutrients

4.3.5.1. Nutrient status in leaves (Table 42)

Potassium content (%)

There was no significant differences in potassium content in both the seasons among the treatments, and the potassium contents varied negligibly between 1.03 and 1.04 per cent.

Calcium content (µg g^{-1})

Giving chemical and growth regulator sprays on TS 29 plants during panicle development significantly resulted in reduced calcium contents of leaves analysed during booting stage of both the seasons as compared to untreated control plants, which recorded maximum calcium contents of 208.39 and 203.18 µg g^{-1} during season I and season II respectively. Among the chemicals and growth regulators, very low calcium content was observed in salicylic acid 800 ppm followed by salicylic acid 1000 ppm in both the seasons. Both ethrel and maleic hydrazide treatments performed significantly better than salicylic acid treatments. It appears that salicylic acid sprays, either at 800 or 1000 ppm concentration, during panicle development interfered with calcium nutrition of leaves.

Copper content (µg g^{-1})

Similar to calcium content, copper content also was distinctly low in salicylic acid treatments which ranged between 81.50 and 86.21, whereas in control plants, the copper contents ranged from 117.42 to 118.61 µg g^{-1} during both the seasons. Both ethrel and maleic hydrazide performed intermediate to control and salicylic acid treatments. The study revealed that salicylic acid treatment explicitly interfered with copper nutrition of leaves.

Boron content (µg g^{-1})

Significantly poor values were exhibited by salicylic acid treatments with regard to boron content also, which ranged between 20.42 and 23.22, while the boron content values in control plants were 38.49 and 38.82 during season I and season II respectively. Both ethrel and maleic hydrazide treatments exhibited slightly increased boron content values over salicylic acid treatments. The distinct difference between values of salicylic acid treatments and control showed that salicylic acid restricted the boron accumulation in leaves.

4.3.5.2. Nutrient status in panicles (Table 43)

Potassium content (%)

Unlike leaves, the panicles of TS 29 revealed significant differences among treatments in respect of potassium content with control plants recording potassium content of about 0.8 per cent during both the seasons. Salicylic acid 800 ppm treatment resulted with poor potassium contents in panicles with a range of 0.46 to 0.59 per cent. But, maleic hydrazide 2000 ppm revealed better potassium status of 0.67 and 0.72 per cent during season I and season II respectively followed by ethrel 800 ppm. Obviously, salicylic acid restrained the potassium movement to panicles, and maleic hydrazide and ethrel appeared to be less deleterious in this aspect.

Calcium content (µg g^{-1})

Untreated control outbeated all other treatments by showing high calcium content values of 177.65 and 180.5 µg g^{-1} during both the seasons. On the contrary, salicylic acid 1000 ppm recorded very low calcium content values of 123.53 and 108.35 during season I and season II respectively. Ethrel and maleic hydrazide treatments almost performed uniformly with better calcium contents than salicylic acid treatments. Among the chemicals and growth regulators tried, salicylic acid treatment had definite impact by reducing calcium content of panicles substantially.

Copper content (µg g^{-1})

The chemicals and growth regulators were found to lower copper content of panicles of TS 29, as compared to control plants. The content in untreated control was about 90.8 ,whereas in other treatments, the contents were well below 73 µg g^{-1}. Very low contents of 65.87 and 60.76 µg g^{-1} were recorded in salicylic acid 800 ppm during season I and season II respectively. Ethrel and maleic hydrazide treatments were comparatively better than salicylic acid treatments in terms of copper contents. Distinct reduction in copper content of panicles by salicylic spray was clearly established in the study.

Boron content (µg g^{-1})

Nearly 50 per cent reduction in boron contents over that of control plants was observed in chemical and growth regulator treatments. In control plants, boron contents ranged between 31.7 and 33.0 µg g^{-1}. In other treatments, the contents were below 17.31, among which, salicylic acid treatments performed poorly with a range of 14.22 to 15.72. Boron being an important nutrient for pollen fertility, the steep reduction in the content, caused by salicylic acid spray, might have resulted in sterility.

4.4. EFFECT OF VARIOUS PHENOLS ON INDUCTION OF STERILITY IN TS 29

Under Glass house condition, eight different phenols were sprayed at 1000 ppm concentration, each at stage III and stage V of panicle development in TS 29. Percentages of pollen and spikelet sterilities were recorded and the data are furnished in Table 44. The treatments were compared with untreated control, which recorded 13.31 per cent pollen sterility and 16.51 per cent spikelet sterility. Significant differences were revealed by the treatments with salicylic acid recording very high pollen sterility of 98 per cent and spikelet sterility of 99.5 per cent followed by catechol (90.5 and 92.5 per cent respectively). Cinnamic acid, pyrocatechol and chlorogenic acid also effected

more than 75 per cent pollen and spikelet sterilities. Among the phenols, very low pollen and spikelet sterility values, ranging between 50 and 61 per cent, were observed in syringic acid and ferulic acid.

Out of the eight phenols tested under Glass house condition, salicylic acid, cinnamic acid, catechol and pyrocatechol were selected and tested under field conditions by spraying these phenols at 1000 ppm concentration each on TS 29 during stages III and V of panicle development. Pollen sterility (%) and spikelet sterility (%) data are furnished in Table 45. In untreated control plants, pollen and spikelet sterility were 15.8 and 16.5 per cent respectively. But, salicylic acid was found to effect maximum pollen sterility of 97.5 per cent, and maximum spikelet sterility of 98.5 per cent followed by catechol with 88.5 and 90.1 per cent respectively. Pyrocatechol recorded very low pollen and spikelet sterility of 65.5 and 70.0 per cent respectively. Whereas, cinnamic acid performed moderately better with 80.3 per cent pollen sterility and 85.5 per cent spikelet sterility.

4.5. EFFECT OF CERTAIN CHEMICALS AND GROWTH REGULATORS ON INDUCTION OF FERTILITY DURING STERILE PHASE IN TS 29

Brassinolide (BR) at one ppm, Naphthalene acetic acid (NAA) at 50 ppm, Indole acetic acid (IAA) at 50 ppm and Ascorbic acid at 100 ppm concentrations were sprayed during partial sterile phase at stages III and V of panicle development of TS 29, under both Glass house and field conditions. The treatments were compared with untreated control.

4.5.1. Pollen fertility and seed set (%)

Under Glass house condition, BR (1 ppm) was found to effect very high pollen fertility to the level of 63.3 per cent, and this treatment also recorded maximum seed set of 51.4 per cent. IAA (50 ppm) followed suit with 56.4 per cent pollen fertility and 40.6 per cent seed set. Ascorbic acid was only marginally effective with 25.4 per cent pollen fertility and 21.2 per cent seed set. The values exhibited significant differences among the treatments (Table 46). The data favoured the use of BR at one ppm concentration for producing a modest pollen fertility of 63.3

per cent and a seed set of 51.4 per cent in TS 29 ,when the temperature prevailing during the sensitive phase is sufficient to cause drastic pollen sterility with no seed set. This technique assumes importance, as induction of pollen fertility is also important in thermosensitive rice lines, when the sensitive phase coincides with lethal temperatures. In untreated plants of TS 29, the pollen fertility was as low as 5.4 per cent with absolutely no seed set.

The above treatments were also tested for fertility induction in TS 29 under field condition (Table 47). Brassinolide spray at one ppm concentration recorded significantly high pollen fertility of 40.21 per cent that resulted in seed set of 36.72 per cent. The next best treatment was IAA 50 ppm spray, which recorded 30.26 per cent pollen fertility and 28.39 per cent seed set. NAA (50 ppm) and Ascorbic acid (100 ppm) treatments gave only moderate effects on pollen fertility and seed set. Untreated control plants recorded only 2.95 per cent pollen fertility with absolute absence of seed set. When TS 29 was grown during the season of partial sterile phase that prevailed during panicle development, use of Brassinolide at one ppm appears to be beneficial for effecting pollen fertility and moderate seed set.

4.5.2. Biochemical constituents

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

Use of chemicals for fertility induction in TS 29 line appeared to increase soluble protein content of leaves of TS 29 during panicle development and flowering stages. Application of BR at one ppm concentration during III^{rd} and V^{th} stages of panicle development significantly increased soluble protein content over other treatments and the content increased from 11.14 during early panicle development stage to 14.95 mg g^{-1} during flowering stage (Table 48). Only at flowering stage, both BR and IAA treatments were having equal soluble protein contents (14.95 mg g^{-1}). NAA and IAA each at 50 ppm concentration also produced better soluble protein contents as compared to control plants in which, the content was very low at any sampling stage.

4.5.2.2. Starch content (mg g^{-1})

Reduced starch content values were observed in spray treatments intended for enhancing fertility, especially during panicle development stages when only the control treatment recorded high starch contents of 35.94 and 39.93 mg g^{-1} in two sampling intervals during panicle development (Table 49). Among the spray treatments, ascorbic acid (100 ppm) showed increased starch contents, while all others resulted in almost uniformly low starch contents. At flowering stage, both control and Ascorbic acid treatments revealed almost uniform starch contents of 40.05 and 40.06 mg g^{-1} respectively, when IAA (50 ppm) recorded the lowest starch content of 31.76 mg g^{-1}.

4.5.2.3. Total carbohydrates (mg g^{-1})

Five days after first spray during stage III, NAA (50 ppm) was able to increase the total carbohydrates content significantly upto 88.15 mg g^{-1}, when the control plants were having only 81.34 mg g^{-1} (Table 50). But, after second spray during stage V of panicle development, IAA (50 ppm) registered high total carbohydrate content of 98.84. Very high total carbohydrates content in this treatment was exhibited during flowering also (99.16). In every stage of sampling, control plants maintained lower carbohydrate content than other treatments. NAA and BR treatments also facilitated increase in total carbohydrate contents in every stage of sampling.

4.5.2.4. Proline (mg g^{-1})

Giving Brassinolide sprays at one ppm concentration during stage III and V of panicle development absolutely resulted in the production of lower level of proline in the leaves of TS 29 in all the three stages of sampling. The data (Table 51) revealed significant differences among treatments. In control plants, proline contents were remarkably very high in every stage of sampling as compared to chemical treatments. Ascorbic acid at 100 ppm was found to effect decrease in proline production only to a limited extent among the chemicals and growth regulators tried. The proline content tended to increase with the advancement of phenological

phases from panicle development to flowering. The results signify the definite role of brassinolide treatment in containing proline production.

4.5.2.5. Total phenols (mg g^{-1})

Data on phenol contents of leaves revealed significant differences among treatments in every stage of sampling (Table 52). However, the differences widened with the advancement of age of the plant. During early panicle development, very low phenol content was observed in BR treatment (2.04 mg g^{-1}). But, during late panicle development stage, IAA (50 ppm) treatment recorded the lowest phenol level of 2.45 mg g^{-1} among the treatments. At flowering, control plants showed very high phenol content of 4.15 mg g^{-1}, when all other spray treatments were having phenol contents below 2.84 mg g^{-1}, with the lowest content of 2.65 mg g^{-1} in BR treatment. The results indicated that the chemicals that favoured fertility were found to minimize phenol production to remarkably decreased levels.

4.5.3. Enzyme activities

4.5.3.1. IAA oxidase activity (µ moles of unoxidised auxin g^{-1} hr^{-1})

IAA oxidase activity values ranged between 15.7 in NAA 50 ppm treatment during early panicle development and 17.45 in the same treatment during flowering stage. The data furnished in Table 53 failed to indicate any clear difference among the treatments. However, control treatment exhibited relatively higher values of IAA oxidase activity during late panicle development and flowering stages. Both NAA and IAA at 50 ppm concentrations maintained reduced activities during these two stages.

4.5.3.2. Nitrate reductase activity (µ moles NO_2 g^{-1} hr^{-1})

Clear and significant differences in nitrate reductase activities were manifested by NAA (50 ppm) treatment in all the three stages of sampling, the values of which ranged from 0.395

to 0.644 (Table 54). Low activity was observed in control plants in every corresponding stages, which ranged from 0.314 to 0.545. The other treatments produced intermediate results between these two treatments. It is apparent that the chemical treatments, meant for increasing fertility, favourably enhanced the enzyme activity.

4.5.3.3. Catalase activity (Enzyme units x 10^4 g^{-1} min^{-1})

Data on catalase activity in leaves of TS 29 showed significantly increased activities in plants that received IAA spray at 50 ppm concentration during all the stages of sampling, namely third (2.98) and fifth (4.01) stages of panicle development, and during flowering (4.24) (Table 55). Brassinolide (1 ppm) spray came next with catalase activity values of 3.96 during late panicle development and 4.13 during flowering stages. NAA (50 ppm) concentration also performed well, besides the above treatments. Only during late panicle development stage, it recorded the lowest value among the treatments.

4.5.3.4. Peroxidase activity (Enzyme units l^{-1} hr^{-1})

With regard to peroxidase activity, Brassinolide treatment recorded remarkably increased activities among all the treatments, in all the three sampling stages, followed by IAA (50 ppm). Very low activity was noticed in control plants. Though the data showed significant differences, the variations among the treatments were not very wide. At flowering, the peroxidase values ranged from 185.43 in control to 193.33 in BR (1 ppm) treatment (Table 56).

4.5.3.5. Superoxide dismutase activity (Enzyme units mg^{-1} protein hr^{-1})

Superoxide dismutase activities were found significantly high in plants that received chemical and growth regulator treatments. Lower values were recorded in control treatments than other treatments. The differences between control and other treatments widened as the plants approached flowering stage (Table 57). SOD activity during early panicle development stage ranged between 7.17 (control) to 8.64 (Ascorbic acid), while during late panicle

development stage, the range was from 6.95 in control to 8.75 in Ascorbic acid treatment. During flowering also the same trend continued, but with wider difference of 6.04 in control and 8.56 in Ascorbic acid treatment. The other chemicals and growth regulators almost performed uniformly like Ascorbic acid treatment with narrow, but significant differences.

CHAPTER V

DISCUSSION

The ever increasing population world over, especially in South East Asian countries, where the food habit of the people is rice oriented, has ushered in an urgent need for planning and executing result oriented achievements for increasing the production of rice to meet the demand in the near future. Agricultural scientists, involved in various rice based research activities, have diverted their attention towards new avenues for enhancing the current rate of productivity, besides sustaining the accelerated rate in rice production. Introduction of hybridization technology, to a large extent, has been identified as a vital and prospective avenue for increasing rice production, and to break the plateau in yield increment encountered in recent years. China has shown the way among the South East Asian countries with successful strides in this direction. India is following suites in this direction with concerted efforts.

In hybridization programme, Environment Sensitive Genic Male Sterility (EGMS) technology is gaining popularity due to the sensitiveness of certain rice lines to the environment, in terms of induction of male sterility. A particular range, duration or concentration of an environmental factor, given at a sensitive stage of phenophase, induces male sterility in certain sensitive lines. Using this technique as a tool, large scale production of hybrid seeds has been attempted in rice, since the manual operation for crossing and hybridization activities is cost prohibitive.

Among the environmentally controlled sensitive lines, in the temperature regulated Thermosensitive Genic Male Sterility (TGMS) lines, the male sterility has been identified to be controlled by a single recessive, heritable nuclear gene, the expression of which is influenced by temperature at a specific stage of panicle development. Falling in line with many research workers, who accelerated their activities towards this area of research in other places, efforts also have been undertaken in Tamil Nadu to popularise hybrid rice production programme. The present investigation was aimed to characterise the thermosensitive nature of an amenable rice line in terms of physiological and biochemical parameters and to identify the particular sensitive stage of rice phenophase for effective male sterility induction, to fix the critical sterility temperature (CST), and to ponder over the possibility of using chemicals and growth regulators to manipulate fertility/sterility induction with a view to support the hybridization programme in rice.

TGMS technology can be best exploited in regions where the temperature variations within a season or year are fairly wide. High temperature above the critical sterility point (CSP), and low temperature below the critical fertility point (CFP) can occur in a year, so that, the breeders can exploit this environmental facility both for production of hybrid seeds during the period of male sterility inducing high temperature regimes, and for multiplication of seeds of parental lines during prevalence of low temperature regimes within a year. Coimbatore is one such place, where, such environmental feasibility is available. The line TS 29 is amenable to pollen sterility/fertility variations under Coimbatore temperature conditions.

Six different experiments were conducted in the present investigation involving the TGMS line, TS 29 and wherever necessary, a known thermo insensitive variety, CO 46 has been involved as a check for comparison. The results are discussed in this chapter under appropriate headings.

5.1. THERMOSENSITIVITY AND ATTENDANT CHANGES DURING REPRODUCTIVE PHASE

TGMS system involves transformations at genic level to influence sterility/ fertility of pollens, as among the reproductive organs pollens appear to be more sensitive to temperature variations,especially during the sensitive stage of panicle development (Sun *et al*., 1989). In India, temperature induced male sterility in rice was reported in several rice lines (Ali, 1993; Ali *et al*., 1995; Sathyanarayana *et al*., 1995; Pandey *et al*., 1998). Jauhaur Ali *et al*. (2000) observed that many TGMS lines manifested complete male sterility when maximum temperature exceeded 30^{o}C, and minimum temperature exceeded 24^{o}C. The same lines produced fertile pollens when maximum and minimum temperatures were 24^{o}C and 16^{o}C respectively. Virmani and Voc (1991) reported of complete pollen sterility at maximum temperature range of 30^{o}C to34^{o}C and minimum temperature range of 22^{o}C to 23^{o}C, while partial to normal pollen sterility was exhibited at lower temperature regimes of 24^{o}C to 27^{o}C / 18^{o}C to 19^{o}C. Not all the lines behaved uniformly to temperature alterations. But for TGMS lines, the mean critical sterility temperature ranged between 23^{o}C to 29^{o}C as reported by Yuan (1992).

5.1.1. Critical sterility temperature (CST)

To identify the exact sterility temperature of TS 29 line, Phytotron study was taken up and different temperature regimes were maintained in the Phytotron during the panicle development stage. The study clearly indicated that for every 0.5^{o}C increment in mean temperature, the pollen sterility was steeply increased above mean temperature of 24^{o}C, and as the mean temperature crossed 25.5^{o}C, the sterility rate raced towards near totality. At 27.5^{o}C and above, 100 per cent pollen sterility was observed in TS 29 line. The study has clearly indicated that mean temperature of 27.5^{o}C is the minimum critical sterility temperature for achieving complete pollen sterility in TS 29 (Fig.3). Any temperature regime of day and night temperatures which works out to the mean temperature of 27.5^{o}C during panicle development of TS 29 appeared to result in complete male sterility. Latha (2001) reported earlier that complete pollen sterility in TS 29 under field condition could be achieved when the mean temperature ranged

between 28.25^{o}C to 29^{o}C, as there was no control over the interference of several weather factors under field conditions, unlike controlled Phytotron condition. Hence, slightly increased mean temperature of above 28^{o}C has been reported for field environment. Borkakati and Virmani (1996) confirmed that the temperature regime of 34^{o}C/21^{o}C with a mean temperature of 27.5^{o}C was the critical sterility temperature in Norin PL 12 and IR 32364. Virmani (1992) also observed similar results in Norin PL 12, and a CST range of 32^{o}C/24^{o}C for a TGMS line, IR 32364-20-1-3-2B under Phytotron. A mean CST of 26.5^{o}C was reported by Mou *et al*. (1998) for several lines. Under Phytotron condition, Roystephen and Thangaraj (2000) and Senthil (2001) identified CST of 26.5^{o}C in TS 16, and 27.5^{o}C in TS 18. Viraktamath and Virmani (2000a; 2000b) reported that the maximum temperature that prevailed during the sterile phase was more effective in inducing sterility.

Considering the climatic conditions that prevailed at Coimbatore for the past 15 years (Fig.4), TS 29 can be involved as a female parent for hybrid seed production during March to June, when the average temperature is above 27.5^{o}C. By adjusting the sowing dates, so as the thermosensitive panicle development phase coincides and falls during the above period, complete pollen sterility can be achieved in TS 29 and used for crossing with a restorer line.

5.1.2. Critical stage of thermosensitivity

The sensitive stage of a TGMS line is the stage of its growth and development, when it is influenced by temperature to cause sterility/fertility expression more precisely (Virmani and Ilyas Ahmed, 2000). As the pollens are the most susceptible organs in a plant and more sensitive to temperature changes, the stage of development of the panicle has to be the sensitive one for temperature. Deng and Yuan (1998) characterized more than ninety photo and thermogenic male sterile (PTGMS) lines of rice, and found that about 73 per cent of the entries had their sensitive stage between pollen mother cell formation and meiosis, which coincided 10 to 14 days before

heading (DBH), and for 19 per cent of the entries, the sensitive stage was from pistil and stamen formation to pollen mother cell formation (12 to 17 DBH).

In the present investigation, 100 per cent pollen sterility was observed in TS 29, when the CST treatment was given during 0 to 30 DBH, 5 to 25 DBH and 10 to 25 DBH. It is clear from the present study (Fig.5) that the minimum duration of fifteen days from 10 to 25 DBH is the precise thermosensitive stage for effecting 100 per cent sterility in TS 29. This period coincides the duration from the origination of secondary branch primordia, when the young panicle is about one mm long and covered with white hairs (20 to 26 DBH) till the filling phase of pollen and floret, when the panicle reaches full length and the colour turns to green (9 to 11 DBH). According to Rangaswamy (1993), the above duration is between stages III and VII of panicle development in rice. In close conformity with the findings from this study, Yao *et al*. (1995) reported that 5 to 20 DBH was the critical stage in TGMS lines. By close scrutiny of present data, it could be inferred that 92.5 per cent pollen sterility was recorded, when CST was given during 10 to 20 DBH. Though complete sterility was not observed, even the record of high pollen sterility of 92.5 per cent led to the conclusion that the most crucial period and minimum duration could be 10 to 20 DBH in TS 29. However for obtaining complete sterility, it is safer to give thermoperiodic treatment for five more days, namely from 10 to 25 DBH. In tune with the above observation, Yang *et al*. (1995) reported that atleast two stages of thermosensitivity existed in TGMS lines, one at about 20 DBH around the formation of pollen mother cell and another at meiotic stage, which might occur between 12 and 15 DBH. However according to Ali *et al*. (1995) the sensitive stage could vary among genotypes. Cheng *et al*. (1996), Lakshmi Praba and Thangaraj (1999), Gong *et al*. (2000) and Senthil (2001) also observed critical thermosensitive stage in certain TGMS lines similar to present investigation.

5.1.3. Physiological and biochemical changes associated with critical sterility temperature

The manifestations in the expression of pollen sterility, as a result of CST treatment during critical stage, might be due to definite alterations in the internal physiological and biochemical activities of the plant. By studying these changes, causes for male sterility induction in TGMS line may be revealed and the underlying facts may broaden our knowledge about TGMS system. The probable contribution of every factor for the ultimate expression of male sterility is discussed here with sufficient support from earlier studies.

5.1.3.1. Hormonal changes

In the present investigation, the plants of thermosensitive TS 29 and thermo-insensitive CO 46, raised under Glass house condition experienced safer temperature regimes with ambient mean temperature prevailing well below the CST. Hence, both the lines produced fertile pollens alone. But, by giving CST treatment to these two lines under controlled Phytotron condition, the thermosensitive TS 29 produced sterile pollens, while CO 46 producing fertile pollens. About 16.8 per cent reduction in leaf **IAA content** and 9.1 per cent reduction in IAA content of young panicles were observed in TS 29 with sterile pollens under CST treatment as compared to that possessing fertile pollens under Glass house condition. The insensitive cultivar, CO 46 revealed only a negligible reduction of 2.4 per cent in leaf and 0.2 per cent in young panicles by CST treatment, which might not have warranted pollen sterility in CO 46. It appeared that any distinct reduction in IAA contents of both leaves and young panicles might lead to pollen sterility, and the CST treatment might have effected such reduction in the level of this hormone. Still more steep reduction upto 73.1 per cent in **ABA content** was observed in TS 29 producing sterile pollens in leaves and 44.1 per cent in young panicles from that of TS 29 plants producing fertile pollens. But, CO 46 exhibited a small reduction of 7.7 per cent in leaves, but with 2.4 per cent increase of ABA content in young panicles when CST treatment was given. Kahlem *et al*. (1975) and Dauphin - Guenin *et al*. (1980) have proposed the hypothesis that sex genes and sterility determinants act on the endogenous hormonal contents of each sexual type. The report of Shen and Gao (1992) lent support to the present investigation by the fact that the IAA content

reduced in long day induced sterile PGMS lines. They also adduced that the cause for pollen abortion was the reduction in IAA level. Roystephen (1998) also opined that reduced IAA level could be associated with pollen sterility in TGMS lines. The reason for steep reduction in ABA level that caused pollen sterility could not be substantiated with supporting evidence. It appeared that a steep reduction in ABA contents of both leaves and emerging panicles might also lead to sterile pollens. Increased level of ABA might be a combating tool to manoeuvre the adverse effect of CST.

Ethylene release rates (ERR) of emerging panicles of TS 29 increased by two and half times when subjected to CST treatment over that of producing fertile pollens under Glass house condition. The insensitive cultivar, CO 46 exhibited only a narrow difference in ERR values between Glass house and Phytotron treated plants. In TS 29, CST treatment might have inflicted harmful effects leading to pollen abortion and sterility. Ethylene is a known senescing and destabilizing hormone, and the effect of excess ethylene is in expected lines. The key role of ethylene in pollen sterility was given by Tian Chang En (2000). The present study also was in line with that of Li De Hong *et al*. (1996), who observed positive relationship with ethylene release rate and pollen sterility. Excess production of ethylene, causing pollen sterility, was also reported by Luo *et al*. (1993), Zhu and Cao (1997), Tian *et al*. (1999), and Senthil (2001) in certain cytoplasmic male sterile (CMS) and TGMS lines.

5.1.3.2. Gas exchange parameters

Among the environmental factors, changes in temperature are known to influence the rates of gas exchange parameters in leaves, namely photosynthetic rate (P_n), transpiration rate (E) and diffusive resistance (r_s). As temperature is responsible for fertility/sterility variations in TGMS lines, the changes in these parameters in TS 29, producing either fertile or sterile pollens, were critically evaluated in the present study in comparison with the changes in the insensitive CO 46, which was also subjected to similar treatments.

Photosynthetic rate, apparently declined steeply in TS 29 plants subjected to CST treatment which resulted in sterile pollens. The decline in the rate was as high as 36.1 per cent, whereas, it was only 10.4 per cent in CO 46 under CST treatment. Being a thermosensitive line, prevalence of temperature above CST level during the sensitive panicle development stage might have caused adverse reduction in the rate, while CO 46 could have resisted the adverse effect of high temperature. It is apparent that any reduction in current photosynthesis during the vulnerable panicle development stage may deprive the developing panicles from getting sufficient nourishments and weaken the pollen formation. Vani *et al*. (2001) observed that exposure of intact rice plants to elevated temperatures might inhibit photosynthesis at multiple sites. The reduction in photosynthetic rate in sensitive plants could be more severe when the electron transport in PS II was impaired and the important carboxylating enzyme, Rubisco might become less stable and begin to denature above the optimum temperature for a process, as revealed by Maruyama *et al*. (1990). Yoshida (1981) has reported that the optimum temperature for pollen development is far less than that for vegetative growth and the rice pollens were very sensitive to elevated temperatures. He also indicated that the optimum temperature for the reproductive growth varied among cultivars. Roystephen (1998) reported reduction in Rubisco content upto 42 per cent in sterile TGMS lines due to CST treatment. Probably, this reduction in Rubisco might be the reason for steep reduction in P_n rate, as recorded in the present study.

Unlike P_n, the **transpiration rate** (E) was not affected severely by CST treatment, as only 6.7 per cent reduction in TS 29 and 2.6 per cent reduction in CO 46 were recorded. Even this increased reduction in the rate of TS 29 would have adversely affected the gas exchange and reduced P_n activity. It is pertinent to note the revelation of Tanner (1963) that energy balance considerations showed an increase in plant body temperature, if the transpiration of the plant decreased that would lower the latent heat exchange. Hence, definite increase in internal temperature of susceptible TS 29 plants would have weakened the delicate bilayer membrane structure of pollen grains and caused sterility.

As could be normally expected, **diffusive resistance** (r_s) did increase with increase in temperature, but the increase was 14.3 per cent higher in TS 29 due to CST treatment, whereas the increase was only 3.9 per cent in CO 46 after the same treatment. The results indicated that inspite of increased diffusive resistance as a result of elevated temperature, the thermosensitive TS 29 could produce only sterile pollens.

5.1.3.3. Carbohydrate fractions

While producing sterile pollens as a result of CST treatment under Phytotron condition, TS 29 leaves were found to maintain very low **reducing sugar** content of 5.61 mg g^{-1} as compared to that of TS 29 with fertile pollens (7.52 mg g^{-1}). The reduction was to the extent of 25.4 per cent. Under similar conditions, only 1.3 per cent reduction was noted in CO 46.

As the reduction in **total sugars** due to CST treatment was as high as 30.3 per cent, it appears that the reduction is more severe in non-reducing sugars than in reducing sugars (Fig.6). As both reducing and non-reducing sugars pool was seriously affected due to the high temperature CST treatment, the reduction might be attributed to the reduced rate of current photosynthesis during panicle development.

Contrary to reduction in sugar fractions, the CST treatment distinctly promoted the **starch content** of leaves, as there was 38 per cent increase in CST treated TS 29 over that of Glass house grown plants. But, a negligible variation in starch content was observed in CO 46 due to CST treatment (Fig.7). Leaf being a source organ, accumulation of starch and depletion of sugars may not augur well, since the assimilation might have slowed down due to low photosynthetic rate or the assimilate movement would have been blocked and converted to starch. This adverse phenomenon might be one of the reasons for lack of assimilate supply to developing panicles in sterile TS 29 leading to sterility.

While taking into account the content of **total carbohydrates** in leaves, there was 16.1 per cent reduction in CST treated TS 29 plants, as compared to the Glass house raised fertile plants. Despite the increase in starch content, the decrease in total carbohydrates in CST treated TS 29 might be due to reduction in the synthesis of various sugar fractions (Fig.7).

The present investigation has clearly indicated that the contents of sugar fractions were severely affected due to CST treatment in the sensitive TS 29 line. Many reports have indicated that under stress condition, accumulation of sugars could provide osmotic effect and mitigate stress damage in plants, but this favour has been denied to TS 29 under increased CST condition, thereby forcing the line to produce only sterile pollen.

In support of the above finding, Markova and Daskaloff (1976) in pepper and Chauhan and Kinoshita (1980) in sugarbeet observed decrease in reducing sugar in plants producing sterile male anthers. They attributed reduced photosynthesis and reduced supply of assimilates for the decrease in reducing sugar that culminated in male sterility. Peng and Wang (1991) and Roystephen (1998) also observed reduced sugar contents in PGMS lines and TGMS lines respectively, which resulted in pollen sterility. Wang *et al*. (1991a) observed reduction in sucrose content in male sterile rice, and He *et al*. (1993) revealed low activities of major enzymes of sucrose metabolism, namely sucrose phosphate synthase and sucrose synthase as a result of long day treatment in Nongken 58 S rice, that induced sterility.

That CST treated sterile rice lines might accumulate more starch was corroborated by the findings of Peng and Wang (1991) and Lakshmi Praba and Thangaraj (2000).

5.1.3.4. Hill reaction and nucleic acid contents

The rate of **hill reaction** represents the efficiency of photosynthetic electron transport at PS II level, and the reaction was severely hampered by CST treatment in TS 29 (30.9 per cent reduction), as compared to CO 46 (0.8 per cent reduction). The result obviously indicated the impairment of PS II system in sensitive TS 29, which might be the major cause for reduced photosynthetic rate due to

CST treatment in thermosensitive TS 29. As CO 46 resisted the reduction in hill reaction rate under CST treatment, the cultivar was able to produce fertile pollens unlike TS 29. Vani *et al*. (2001), in support of the present finding, indicated loss in PS II photochemistry and an associated loss in the number of functional PS II units in rice leaves when the temperature increased over an optimum level. Tang *et al*. (1994) also observed decreased photochemical activity and inefficient light energy transformations in PS II under sterility inducing conditions in rice. In effect, the assimilatory power ATP and NADPH were observed to be reduced by Xia *et al*. (1993) in long day treated sterile PGMS lines. That the reduced hill reaction rate might produce attendant male sterility was in consonance with the findings of Roystephen (1998), Ma Dehua *et al*. (1999) and Senthil (2001).

Both **RNA** and **DNA** were found severely reduced in leaves of TS 29 subjected to CST treatment as compared to CO 46 under similar condition. The reduction was more severe in DNA contents upto 37.5 per cent than in RNA contents (30.5 per cent reduction) in sterile plants as compared to fertile plants of TS 29. But, CO 46 revealed only marginal reduction in these nucleic acids as a result of CST treatment. Panicle development being a crucial stage when, the reproductive organs are in the process of making, the steep reduction in nucleic acid contents at this stage might definitely undermine the growth of reproductive organs, especially pollens. Kaul (1988) also observed similar reduction of nucleic acids in peas and rice mutants upto 54 per cent under sterility inducing conditions. The association of low RNA content with male sterility was earlier reported by Saini and Davis (1969) in onion, Mian *et al*. (1974) in barley and Chauhan and Kinoshita (1980) in sugarbeet. In sterile PGMS rice lines, reductions in RNA contents ranged between 6.3 and 100 per cent (Yang and Zhu, 1990). Similar decline was also reported by Shu and Chen (1999) in sterile TGMS lines. Under Coimbatore condition, Lakshmi Praba and Thangaraj (2000) also recorded reduced RNA content as a result of CST treatment in TS 16 and TS 18.

5.1.3.5. Biochemical constituents

Changes in the levels of important biochemical constituents of leaves ought to have manifested major changes in the form of pollen sterility in a sensitive line.

A major portion of **soluble protein** in leaves is occupied by RuBP carboxylase, a prime enzyme of carbon fixation in photosynthesis (Noggle and Fritz, 1986), and the estimation of soluble protein content is considered as an indirect measure of the enzyme activity. TS 29 was found to loose about 40 per cent of soluble protein content by CST treatment below that of Glass house raised plants (Fig.8). Loss of soluble protein to this extent might have seriously jeopardized RuBP carboxylase activity of TS 29 by CST treatment resulting in lower photosynthetic rate and reduced assimilate supply with an ultimate effect of pollen sterility. The above observation seems to be correct as there was only a negligible decrease of 0.5 per cent in soluble protein content in insensitive CO 46, which produced fertile pollens even after CST treatment. Liu *et al*. (1993b) also observed similar reduction in soluble protein content in sterile PGMS lines. Many authors (Peng and Wang, 1991; Huang *et al*., 1994, 1996; Roystephen and Thangaraj, 2000; Senthil, 2001) attributed reduced availability of aminoacids and reduced nitrogen assimilation for low soluble protein content which could cause male sterility in TGMS lines.

Luo *et al*. (1988) suggested that **proline content** could be used as an index of male sterility. In the present study, there was more than two times increase in proline content in male sterile TS 29 plants after CST treatment over that of Glass house plants with fertile pollens. CST treated CO 46 also showed about 38 per cent increased proline content (Fig.9), but proline increase to this extent appears to be insufficient to effect pollen sterility. The results of the present investigation are in accordance with the findings of Elsy (1997), Roystephen (1998), and Senthil (2001). Even as early as 1973, Khan observed accumulation of proline and hydroxy proline in male sterile plants, but later Kaul (1988) contradicted by observing a decreased level of proline in male sterile anthers. Xiao *et al*. (1993) also reported reduced proline content in sterile anthers of PGMS lines.

Total phenols normally produced as metabolic byproducts when a plant experiences adverse stress situations, and according to Rodionova *et al*. (1995), an association existed between phenolic

compounds and long day and high temperature conditions that were adverse to normal functioning of plants. In the present investigation, 41.6 per cent increase in total phenols was observed in CST treated TS 29 leaves over that of Glass house grown plants, which experienced temperature regimes below CST, but CO 46 failed to reveal increased phenol contents as a result of CST treatment (Fig.10). Roystephen (1998), and Lakshmi Praba and Thangaraj (2000) observed similar results in certain TS lines. The accumulation of such secondary metabolites might be at the cost of normal metabolic substrates intended for individual organ development and differentiation during the course of growth. The shift in the direction of metabolic activity might have caused deficiency in certain areas of development. In the present case, this shift would have resulted in poor assimilate supply to developing pollens causing sterility.

CST effect was distinctly felt in terms of low **total free amino acid** content. Very wide differences in the contents were recorded between fertile and sterile TS 29 with 55 per cent reduced free aminoacid content in CST treated sterile TS 29 plants. As only negligible reduction of 2.8 per cent was recorded, pollens of CO 46 were not affected (Fig.11). The present result is in consonance with that of Peng and Wang (1991). Wang *et al*. (1993) reported that the reduction in free aminoacid content started from the secondary branch primordia differentiation stage in sterile PGMS lines. In TS 16 and TS 18, Roystephen (1998) observed reduced aminoacid pool as a result of CST treatment.

Ascorbic acid forms part of antioxidant system of plants in the combination of glutathione. Liang *et al*. (1995) reported an increase in ascorbic acid and glutathione contents in anthers during fertile phase of PGMS lines. In the present study, a reduction of 35.5 per cent ascorbic acid content in CST treated TS 29 plants might have affected the functioning of antioxidant system with ultimate production of sterile pollens. This observation gains support from the fact that only a negligible reduction of 2.1 per cent ascorbic acid content was noted in CO 46 after CST treatment and hence, the pollen fertility was not affected in this cultivar. Roystephen and Thangaraj (2000) opined that consequent to CST treatment, the reduced

production of ascorbic acid might have caused accumulation of free radicals, which in turn, was responsible for causing pollen abortion in TGMS lines.

5.1.3.6. Enzyme activities

The results obtained from the present investigation revealed that the unoxidised auxin, after the activity of **IAA oxidase,** was high in TS 29 producing fertile pollens (15.62 µ moles g^{-1} hr^{-1}), whereas, the value was 9.31 in the leaves of TS 29 producing sterile pollens due to CST treatment (Fig.12). The obvious reason for reduced level of unoxidised auxin in sterile TS 29 might be due to high IAA oxidase activity that reduced the auxin content as a consequence. With only limited reduction of unoxidised auxin in CST treated CO 46, the little reduction in IAA content in CO 46 did not warrant the production of sterile pollens. As the genes controlling maleness was correlated with auxin content, the increased activity of IAA oxidase might have reduced the auxin content in male sterile lines (Hamdi *et al*., 1987). This could be the reason for male sterility in TS 29 also as Paulas and Shanmugavelu (1988) observed reduced activity of IAA oxidase during reproductive phase so as to enable optimum supply of auxin for flowering process. The increased activity of IAA oxidase during the stage of panicle development might have adversely affected the reproductive growth of TS 29. Xiao *et al*. (1993) and Roystephen (1998) also observed similar increase in IAA oxidase activity in sterile PGMS lines.

Nitrate reductase is a major enzyme in the biochemical processes of a plant. In the present study, drastic reduction in activity to the extent of 41.4 per cent in CST induced male sterile TS 29 plants (Fig.13) would have destabilized the normal metabolic activity during panicle development resulting in sterile pollens. It is to be noted that there was no such reduction in check cultivar, CO 46, which produced only fertile pollens after CST treatment. Shen and Gao (1992), and Roystephen and Thangaraj (2000) reported that nitrate reductase activity was highly sensitive to temperature fluctuations, and the reduction in the activity could have adversely affected nitrogen assimilation and

amino acid synthesis, which in turn, might have caused depletion of free amino acid pool in cytosol of male sterile plants.

About two and half times increased **ascorbic acid oxidase (AAO)** activity in male sterile TS 29 over male fertile plants suggested that the activity might have seriously depleted the ascorbic acid content (Fig.14), thereby preventing the plants from the benefit of performing antioxidant activity under adverse environments. The increased ascorbic acid oxidase activity might be one of the several reasons for causing pollen sterility in TS 29, when temperature was above CST level for TS 29. Though 41 per cent increased AAO activity was also recorded in thermo-insensitive CO 46 after CST treatment, increase of activity to this extent might not be sufficient to inflict damage in the pollens. Lakshmi Praba and Thangaraj (2000) and Senthil (2001) also observed similar increase in AAO activity in certain male sterile TGMS lines after CST treatments. But, Liang *et al*. (1995) reported contradictarily by observing reduced AAO activity in anthers with fertile pollens. However, according to Zhang *et al*. (1994a), both AAO and glutathione reductase activities increased in PTGMS rice lines after sterility induction.

Glutamate dehydrogenase (GDH) catalyses the reversible reaction that synthesise or deaminates glutamate. Tang *et al*. (1994) reported that the reduction in GDH activity was due to marked decline of photochemical activity of chloroplast with lower PS II activity and impaired NADP reduction. According to Zhu and Yang (1992), the reduction in activities of dehydrogenases like GDH, under long day and high temperature conditions, affected the fertility percentage of pollens. In the present study, the GDH activity was as low as 33.3 per cent in male sterile TS 29 from that of male fertile TS 29 plants. This severe reduction in CST treated TS 29 might have seriously affected the photochemical activities of chloroplasts, whereas CST treated CO 46 maintained 79.3 per cent activity of that of non-CST treated CO 46 and avoided the incidence of male sterility.

5.1.3.7. Activities of active oxygen species scavenging enzymes

For maintaining healthy metabolic system in any living organism, the scavenging of active oxygen species that were produced during the course of metabolic activities is essential. The activities of enzymes, involved in such scavenging, might influence the nature of sterility/fertility changes. Both thermosensitive TS 29 and thermo insensitive CO 46 were grown in Glass house without CST treatment and in Phytotron with CST treatment during panicle development stage. The changes in activities of important scavenging enzymes in CST induced male sterile and male fertile plants are discussed here under.

In male sterile TS 29, **catalase activity** was reduced more than 50 per cent as that of fertile TS 29. But the activity was reduced by about three per cent in CST treated CO 46 and so, only fertile pollens were produced. Very low catalase activity in CST treated TS 29 plants would have enhanced the levels of reactive hydroxyl radicals that might have caused peroxidation of unsaturated lipids of cell membrane in fragile pollen cells and damaged the structural integrity. Similar decreases in catalase activity were reported by Shen and Gao (1992); Zou *et al*. (1993) and Zhang *et al*. (1994a) in sterile EGMS lines. Roystephen and Thangaraj (2000) also observed pollen abortion in TGMS lines due to reduced catalase activity.

The major function of **peroxidase** is catalytic conversion of peroxide to water, thus eliminating the oxidative damage, lipid peroxidation and denaturation of proteins (Bowler *et al*., 1992). In sterile TS 29 line, about 26 per cent reduction in peroxidase activity was observed in the present study from that of fertile plants. But, in the resistant CO 46, a narrow decrease of 6.5 per cent peroxidase activity was recorded due to CST treatment. Roystephen and Thangaraj (2000) in TGMS rice, Cheng and Xiao (1993) in PGMS rice, Zhang *et al*. (1994a), and He and Xiao (1993) in Nongken 58 S rice also observed similar decreases in peroxidase activity that ultimately resulted in male sterility.

Superoxide molecules are to be catalysed to hydrogen peroxide by **superoxide dismutase** (SOD) and the hydrogen peroxide, in turn, to water by peroxidase. Zou *et al*. (1993)

reported that the SOD activity was important in plants to tolerate high light intensity and higher temperature situations. In higher plants, the significance of SOD activity as a protective enzyme has been emphasised by Bowler *et al*. (1992). He and Xiao (1993) observed a close association between SOD activity and pollen fertility in Nongken 58 S. In the present study, the sensitive TS 29 producing sterile pollens after CST treatment, was found to have only 52.3 per cent SOD activity as that of plants having fertile pollens. Apparently, the CST treatment has not affected CO 46 to the extent of causing male sterility, as the decrease in activity due to CST treatment was minimal (4.3 per cent). Such reduced SOD activity was also observed by Zhang *et al*. (1994a), Roystephen (1998) and Senthil (2001) in certain TGMS lines.

The results of this study clearly indicated that a steep reduction in catalase, peroxidase and SOD activities, as a result of CST treatment, could induce male sterility in TS 29. Lower activities of these enzymes facilitated accumulation of active oxygen species and harmed the sensitive structures like pollens.

5.1.3.8. Nutrient status

Leaves of TS 29 and CO 46, which were grown in Glass house without CST treatment, and in Phytotron with CST treatment were analysed for certain nutrients, as the variations in the contents of these nutrients might provide evidence for fertility/sterility changes due to CST treatment. After CST treatment, the sensitive TS 29 produced sterile pollens, when CO 46 could produce only fertile pollens.

The reduction in leaf **nitrogen** content in CST treated male sterile TS 29 was 17 per cent, whereas, the reduction was only 2.3 per cent in CO 46 after CST treatment. It appeared that the reduced nitrogen content in sterile TS 29 would have deprived the line from getting optimum supply of amino acids affecting the synthesis of proteins and various metabolic enzymes. Peng and Wang (1991) also attributed reduced leaf nitrogen content for sterility in rice. They opined that the cause for sterility could be due to reduced synthesis and blocking of transport of amino

acids or proteins to the developing anthers. In contrast, He *et al*. (1993) and Elsy (1997) failed to notice any difference in nitrogen contents between sterile and fertile plants.

In sterile TS 29, the decrease in **potassium** content was more severe than in nitrogen content, as the reduction in leaf potassium content in sterile TS 29 was nearly 32 per cent from that of fertile plants. Optimum potassium level in any plant is considered as a tool to resist environmental stresses, and hence, the steep reduction in potassium level might be attributed to the susceptibility of TS 29 to elevated temperature producing sterile pollens. When exposed to high temperature, certain bean cultivars were found to have reduced potassium level in leaves (Moreno-Limon *et al*., 2000). Reduction in both nitrogen and potassium contents were also noticed under sterile condition in rice by Dell (1981), and Rerkasem and Jamjod (1997).

Among the micronutrients, namely **calcium, copper** and **boron**, the content of boron was found to reduce drastically in sterile TS 29 as compared to the fertile one. The reduction was as high as about 34 per cent, whereas about 24 and 23 per cent reductions were observed in calcium and copper contents respectively as a result of CST treatment to TS 29. In CO 46, the reductions were only marginal after CST treatment (Fig.15) and hence, produced fertile pollens. Such marginal reductions in the reports on the effects of micronutrient deficiencies causing male sterility in rice have been quoted by Dell (1981), and Rerkasem and Jamjod (1997). Wu and Li (1993) and Jian *et al*. (1998) emphasized the importance of calcium in leaves and young panicles for pollen fertility, as calcium might influence cell functions through spatial and temporal changes induced by stimuli (Bush, 1995). Agarwala *et al*. (1980) and Azouaou and Souvre (1993) found relationship between copper deficiency and male sterility. Boron deficiency induced male sterility has been reported by Garg *et al*. (1979) in rice.

5.2. Relative biochemical changes in TS 29 producing fertile and sterile pollens

5.2.1. Free amino acids

By using paper chromatographic technique, the contents of certain free amino acids in leaves of fertile and sterile TS 29 plants were analysed. Among the seven amino acids studied, the contents of isoleucine and tyrosine were more higher in fertile plants (0.290 and 0.277 mg 100 g^{-1} respectively) than others. Curiously, these two amino acids were totally untraceable along with lysine in sterile lines. Cysteine, methionine and histidine were present in both fertile and sterile TS 29, however with reduced quantities in sterile plants. Leucine showed a negligible presence in sterile lines. But, all the seven free aminoacids were present in fertile lines with a range of 0.137 (methionine) to 0.290 mg 100 g^{-1} (isoleucine). The study revealed that the complete absence of isoleucine, tyrosine and lysine and the negligible presence of leucine might have contributed to male sterility in TS 29. As early as 1967, Whited reported reduced contents of leucine, isoleucine, phenylalanine and valine in the sterile anthers of barley. Similar findings were reported by Kern and Atkins (1972) in sorghum. The total absence or negligible production of certain amino acids may derange the normal metabolic activity in plant system and curtail protein synthesis. Such deficiencies in protein metabolism might have cropped up in male sterile TS 29.

5.2.2. Individual phenols

HPLC profiles of six phenolic compounds in leaves of sterile and fertile TS 29 plants revealed that salicylic acid (5.34 mg g^{-1}), catechol (1.73 mg g^{-1}) and pyrocatechol (3.61 mg g^{-1}) were present in the leaves of sterile plant, while others were not in detectable quantities. But in leaves of fertile plants, none of these phenolic compounds was detected. The study indicated that salicylic acid, catechol and pyrocatechol, either individually or collectively, might have contributed for pollen sterility. In this aspect, Srivastava *et al*. (2000) observed thirteen phenols in photosensitive and ten phenols in photoinsensitive rice cultivars. That the presence of higher amounts of phenols interfered with respiratory activity and inhibited oxidative phosphorylation and ion transport was earlier indicated by Demos *et al*. (1975).

5.2.3. Individual polyamines

HPLC profiles of the polyamines spermidine, putrescine and cadaverine in leaves and emerging panicles of male fertile and sterile TS 29 line revealed that the leaves of fertile plant were found to have high content of spermidine, which was absent in leaves of sterile plants. On the contrary, putrescine was absent in fertile plant leaves, when sterile plant leaves showed high content. But, cadaverine was present in both the leaves with slightly increased contents in fertile plants. Unlike the leaves, the panicles of fertile plant were found to have a low spermidine content and that of sterile plants having a low content of putrescine, the cadaverine being absent in both. It appeared that the presence of spermidine favoured fertile pollens, while putrescine favouring sterile pollens. Vernon *et al*. (1993) indicated that plants tended to produce low molecular weight metabolites in response to environmental stresses. Feng and Cao (1993) also observed increased putrescine content under sterility and increased spermidine content under fertility conditions in C407S rice.

5.3. Effect of chemicals and growth regulators on sterility induction in TS 29

For an effective hybridization programme in rice, the availability of temperature regimes above critical mean temperature in a season is helpful for effecting complete sterility. However, such high temperature regimes may not be available throughout the year. During cooler months partial sterility may occur, which may hamper hybridization programme. Under such circumstances, use of chemicals or growth regulators for inducting male sterility without affecting the female organs may be of much use in hybrid seed production. Chemicals like salicylic acid, ethrel and maleic hydrazide, when used in optimum concentration, have been found to induce sterility in sensitive rice lines. The present investigation has been taken up to study the effects of these chemicals on the extent of pollen sterility in TS 29. With this purpose, TS 29 rice plants were raised under field conditions in two seasons, when the temperature

regimes during the sensitive panicle development phase were below the critical sterility temperature, and only partial sterility could be obtained.

5.3.1. Weather conditions during the experimental period

The weather conditions that prevailed during first season (Rabi 2001-2002) and second season (Kharif, 2002) are furnished in Table 10 and 11. As the panicle development stage being the critical stage of thermosensitivity (0-30 DBH), the stage for first season coincided between 4th February and 4th March of 2002, during which period, the mean temperature ranged between 24.8^{o}C and 26.2^{o}C, which was well below the critical sterility temperature of 27.5^{o}C for TS 29. The maximum temperature ranged between 33.2^{o}C and 29.8^{o}C, while the minimum temperature was between 17.6^{o}C and 21.0^{o}C. In the second field experiment conducted during Kharif, 2002, the panicle development stage had fallen between 29th August and 30th September. The mean temperature during this period was between 26.9^{o}C and 25.9^{o}C. The maximum temperature ranged between 32.8^{o}C and 28.7^{o}C, whereas the range of minimum temperature was from 21.1^{o}C to 23.1^{o}C. Hence, during both the seasons, the mean temperature during the critical panicle development stage was below the critical sensitive temperature of 27.5^{o}C. According to Sun *et al*. (1989), and Virmani and Voc (1991), TS lines generally expressed complete pollen sterility at temperature regimes with 30^{o}C – 34^{o}C/21^{o}C-23^{o}C maximum and minimum temperatures respectively. Below these temperature regimes, only partial pollen sterility was reported. Under Coimbatore field conditions, Latha (2001) reported a mean temperature range of 28.25^{o}C to 29^{o}C as the critical sterility temperature for TS 29. In the present study, under controlled conditions CST of 27.5^{o}C has been identified as reported earlier. Hence, for testing the efficacy of salicylic acid, ethrel and maleic hydrazide treatments, the sowings were so adjusted as to make the panicle development stage fall during the periods when the mean temperatures were well below the CST level of 27.5^{o}C.

5.3.2. Pollen sterility

By giving salicylic acid spray at 1000 ppm concentration, a near complete pollen sterility was obtained. But, salicylic acid treatment at 800 ppm has also produced comparable results with slightly lower values. Ethrel and maleic hydrazide also produced pollen sterility averaging between 81.0 and 82.0 per cent (Fig.16). But, for obtaining complete pollen sterility, which would be conducive for effective hybridization programme in TS 29, salicylic acid spray at 800 ppm concentration could be adopted. Salicylic acid treatment was reported to promote cyanide-resistant respiration in mitochondria, thereby increasing the tissue temperature (Taiz and Zeiger, 2002). In this regard, Raskin *et al*. (1987) revealed that application of salicylic acid at 0.13 µg g^{-1} of fresh weight to the sections of immature spadix of Arum lilies led to a temperature increase of about 14^{o}C above the ambient temperature. The induction of near complete pollen sterility in the present investigation with salicylic acid might be due to the increase in body temperature of the plant that showed parallelism to CST treatment in induction of sterility.

5.3.3. Crossed seed set percentage

In hybridization programmes, the chemicals used for induction of male sterility should not harm the female organs. For knowing the effect of these chemicals on the fertility of female organs, restorer lines were also raised along with TS 29 to study the percentage of crossed seed set as an indirect way of knowing the female fertility. In both the seasons, maximum percentage of crossed seed set was observed in salicylic acid at 800 ppm in both rope pulling and clipping methods of crossing. Although salicylic acid 1000 ppm also recorded comparable results, the lower dose of 800 ppm could be adopted for getting higher crossed seed set. As the crossed seed set percentages recorded in ethrel and maleic hydrazide treatments were far below that of salicylic acid treatments, it appeared that these chemicals would have affected the female organs to a certain extent. So also, the partially sterility inducted pollens from these chemical treatments might have also interfered in the hybridization with pollens of restorer lines. Ali (1996) emphasized the need for obtaining stable male sterility by a simple chemical treatment without

any accompanying adverse effect on female fertility in two line breeding using TGMS system. Salicylic acid seemed to be the appropriate chemical for achieving this goal. The effectiveness of salicylic acid in achieving near complete male sterility without affecting the female fertility in TGMS lines was also reported by Lakshmi Praba and Thangaraj (2000), and Senthil (2001). The usefulness of ethrel in inducing male sterility in rice was indicated by Aswathanarayana and Mahadevappa (1991) in rice, Rowell and Miller (1971) in wheat, and Thakur and Rao (1988) in pearl millet. The efficiency of maleic hydrazide in inducing male sterility in cotton was indicated earlier by Singh (1964), in Phaseolus by Kaul and Singh (1967), in wheat by Chopra *et al*. (1960) and in rice by Aswathanarayana and Mahadevappa (1991) and Senthil (2001). That, maleic hydrazide not only caused pollen sterility but also high spikelet sterility was reported by Manjula and Ibrahim (1999).

Fluorescent microscopy study showed that the pollen germination occurred after the pollen of the restorer line MDU 5 reached the stigmatic hairs of TS 29 line treated with salicylic acid. The pollen tube then elongated and passed through the centre hole of the stigmatic hair. In the stigmatic and stylar tissues, the pollen tube passed through the transmitting tissue located in the space between the centre area and the vascular bundle. Finally, the pollen tube reached the bottom micropyle end of the ovary, proving the selective sterility of the chemical (Plate 8). Lasa and Bosemark (1993) reported that a gametocide could emasculate the plants resulting in male sterility while maintaining normal female fertility.

The microtome sections of the bottom of the ovary showed a narrow space between the outer wall of the ovule and the ovary wall. This space showed where the pollen tube passed through the micropyle, confirming the female fertility of the chemically induced TS 29 line (Plate 8). Hattori and Wada (1996) observed conditions of pollen tube elongation on the stigmatic hair and pollen tube entry into the ovary.

5.3.4. Effect of chemicals and growth regulators on certain biochemical constituents

Salicylic acid treatment, either at 800 ppm or at 1000 ppm concentration, effected definite increase in **starch content** of TS 29 leaves and this increase was accelerated with the advancement of age upto flowering. It has been reported elsewhere that CST treatment favoured increase in starch content in TS 29. As similar increase was observed in salicylic acid treatment also, the view that the salicylic acid treatment would increase the body temperature and the induction of male sterility might be due to the manifestation of such increased temperature, gains support from the results. Similar increase in starch content with salicylic acid treatment was also reported by Rajendra and Bates (1981) in wheat, Sharma *et al*. (1992) in mash bean and Senthil (2001) in TGMS rice.

However, **total carbohydrate content** was found to be low in salicylic acid treated TS 29 leaves. This decrease might be due to reduced levels of both reducing and non-reducing sugars, as observed earlier in CST treatment. Being a source organ, the low content of total carbohydrates implicitly indicates the poor source activity of the leaf with reduced assimilate load. Paucity of assimilates, in consequence to salicylic acid treatment at the time of reproductive organ development, might be a cause for pollen sterility. In tune with this proposition, Janda *et al*. (1998) recorded decreased assimilation of carbohydrates in maize due to salicylic acid treatment. Shehata *et al*. (2000) observed reduced content of soluble sugars in cotton after salicylic acid treatment. The reduced carbohydrate content might be coupled with reduced dehydrogenase activities and decreased ATP contents, which in turn might lead to male sterility (Kaul, 1988).

As salicylic acid treatment in TS 29 more or less imitates the effects of CST, not only the carbohydrate fractions but also the amino acid pool might have been reduced by salicylic acid treatment, which finally ended with decreased **soluble protein content**. As the decrease in content became more apparent during flowering, the effect of salicylic acid treatment seemed to persist with enhanced vigour even several days after the treatment. The carboxylating enzyme,

Rubisco occupies major share of total soluble proteins and so, any reduction in soluble protein content is bound to affect the CO_2 fixation and assimilate synthesis. Pancheva and Popova (1998) observed inhibition of soluble protein synthesis and reduction in the activity of Rubisco after salicylic acid treatment in barley. The male sterility induced by pyridoxine derivatives in rice was reported to be closely related to the deficiency of soluble protein during anther development (Zhang *et al*., 1996). Asthana and Srivastava (1977) suggested that the reduction in protein content by salicylic acid treatment might be due to low 'N' utilization in the metabolic stream.

Increment in **proline** content of leaves after salicylic acid treatment at 1000 ppm concentration indicated the stress like effect of salicylic acid treatment almost equivalent to that of CST. The proline increase in salicylic acid treatment was more than three times as that of untreated control, as the panicle development reached flowering stage. Presence of higher amounts of proline was also noticed in ethrel and maleic hydrazide treatments, but not to the extent of salicylic acid treatment. That, the abnormal metabolism of accumulation of free proline during pollen development process could cause sterile anthers, was also indicated by Xiao *et al*. (1988), Lakshmi Praba and Thangaraj (2000). Senthil (2001) also observed proline accumulation due to salicylic acid treatment in certain TGMS lines. The results suggested that the stress like effect of salicylic acid treatment might have caused diversion in normal metabolic process of producing glutamate and other essential amino acids for protein synthesis towards the synthesis of proline. **Total free amino acid** contents in leaves of salicylic acid and other chemical treated plants were low as compared to that of untreated plants which could explain that the free amino acid pool was getting depleted at the cost of proline production. This diversion might lead to reduced enzyme activity and decreased protein synthesis, especially during the crucial stage of reproductive development.

A greater accumulation of **total phenols** after salicylic acid treatments was detected in TS 29, and the contents continued to increase upto flowering. Ethrel and maleic hydrazide treatments were not as effective as salicylic acid in promoting the production of total phenols, which also might be a cause for reduced pollen sterility induction by these chemicals unlike salicylic acid. Among the phenols, salicylic acid treatment strongly favoured the synthesis of benzoic acid and pyrocatechol apart from the synthesis of salicylic acid, which were not detected either in ethrel or maleic hydrazide treatments. But, maleic hydrazide appeared to favour the production of ferulic acid and catechol more than any other treatment. Syringic acid appeared to have no role in induction of sterility as it was not detected. The combination of the above phenols except syringic acid in higher concentrations might have interfered in metabolic functioning to cause pollen sterility. Phenol related thermogenesis was pointed out by Delalonde *et al*. (1996). The present findings concurred with that of Thangaraj and Lakshmi Praba (2000).

It has been pointed out in this chapter earlier that CST treatment could increase the **ethylene release rate** (ERR) of emerging panicles enormously. In salicylic acid treatment, the rate increased by three times as that of untreated plants. This large increase in ethylene production might not have occurred without generating heat in the internal tissues by salicylic acid. This indicated that salicylic acid might effectively increase the ethylene production for causing pollen sterility, as ethylene release rate was reported to show strong positive relationship with pollen sterility in TGMS lines (Luo *et al*., 1993; Li De Hong *et al*., 1996).

5.3.5. Effect of chemicals and growth regulators on activities of certain enzymes

It has been proved beyond doubt that certain chemicals and growth regulators produced pollen sterility in TGMS lines similar to CST treatment, but the extent of sterility varied with the chemicals used. These chemicals would have acted through various enzymes that could have caused an imbalance in normal metabolism leading to pollen sterility.

The present study indicated that salicylic acid and other chemicals could generate perceptible changes in **IAA oxidase activity**, mainly during flowering, as the unoxidised auxin content during flowering was very low. The revelation that the unoxidised auxin content in the leaves of TS 29, treated with salicylic acid, was far lower than that with other chemicals indicated that the auxin content would have been much reduced by salicylic acid, and this reduced auxin level might be responsible for pollen sterility. The present finding is in agreement with that of Thangaraj and Lakshmi Praba (2000), and Senthil (2001), who have studied male sterility in certain TGMS lines. Reduction in IAA content due to increased activity of IAA oxidase by any chemical means might induce male sterility in rice was also indicated by Tang *et al*. (1996). In this context, Huberman *et al*. (1997) adduced that reduced IAA synthesis and impaired auxin transport were the major mechanisms, by which, chemicals caused abnormal reproductive organs. Among the chemicals, salicylic acid appeared to be more effective agent in inducting male sterility by way of reducing the IAA content.

Nitrate reductase, being a dominant enzyme of N metabolism, any reduction in its activity by chemical agents could lead to imbalance in the metabolic stream with adverse effects. In the present study, during panicle development and during flowering, the chemicals that could induce male sterility adversely affected the nitrate reductase activity. Though maleic hydrazide happened to be more deleterious in decreasing the activity of this enzyme, salicylic acid treatment also decreased the activity appreciably. The wide difference in the activity of nitrate reductase between treated and untreated plants indicated the importance of optimum nitrate reductase activity for producing fertile pollens. Contrary to this finding, increased nitrate reductase activity by foliar application of salicylic acid was recorded by Singh *et al*. (2001) and Pramod Kumar *et al*. (1999) in soybean, and Jagadish Rane *et al*. (1995) in wheat. However, the concentrations adopted by them were far lower than that adopted in the present study. Probably, salicylic acid might be a promoter of nitrate reductase activity at low concentrations.

Salicylic acid and other chemical treatments almost doubled the activity of **ascorbic acid oxidase** as compared to that of control plants. This enormous increase in the activity might have depleted ascorbic acid content of the cytosol leading to deficiency in antioxidant system. As a consequence, the unscavenged free radicals might have increased in quantity affecting the developing pollens ending with sterility. Roystephen (1998) concurred this view after recording high ascorbic acid oxidase activity after CST treatment. Senthil (2001) recorded similar results with salicylic acid treatment in certain TGMS lines.

5.3.6. Effect of chemicals and growth regulators on activities of free radical scavenging enzymes

In a biological system, accumulation of waste materials during biochemical reactions in the form of free radicals is bound to happen. But by operating a suitable system, these materials would be scavenged and disposed off to avoid interference to the normal metabolic reactions. Apparently, the free radical scavenging enzymes have to function properly to keep the system clean. Any reduction in the activity of such enzymes might lower the efficiency of metabolic activity leading to complications. The activities of some of the scavenging enzymes, related to sterility/fertility promotion in TS 29, are discussed here under:

The chemicals salicylic acid, ethrel and maleic hydrazide, which have induced male sterility, also were found to reduce catalase activity in the leaves of TS 29. As more pronounced reduction of this activity was in salicylic acid treatment, the chemical appeared to be more effective in reducing catalase activity. Chenzhi *et al*. (1997) reported similar decrease in catalase activity by salicylic acid treatment in rice. That any decrease in normal functioning of catalase could lead to accumulation of reactive oxygen was highlighted by Gehlot *et al*. (1999). The present finding also draws support from Dat *et al*. (1998), and Lopez *et al*. (1998), who studied in mustard and potato respectively.

In line with catalase, peroxidase activity also was considerably lowered by salicylic acid. The reduction in activity by salicylic acid was more pronounced than that of ethrel and maleic hydrazide. Tian *et al*. (1999) observed decreased activities of free radical scavenging enzymes

including peroxidase using ethrel. Dat *et al*. (1998), and Lopez *et al*. (1998) revealed that both catalase and peroxidase activities were affected by salicylic acid treatment.

A wide difference in superoxide dismutase (SOD) activity was recorded during panicle development and flowering stages of TS 29 between chemical treated and untreated plants. But, salicylic acid appeared to be very effective among the chemicals with very low SOD activity. Tian *et al*. (1999) recorded reduced SOD activity with ethrel treatment. As Li Yue Ying and Chen Fen Yu (2001) observed accumulation of peroxide and superoxide molecules with reduced activity of SOD, the salicylic acid induced low activity of SOD in TS 29 would have favoured the free radical accumulation and damaged the tender pollen cells causing sterility, as was evident from the present study.

5.3.7. Effect of chemicals and growth regulators on status of certain nutrients in leaves and panicles of TS 29

Unlike CST treatment, the chemicals, including salicylic acid, failed to influence significant changes in potassium (K) content of TS 29 leaves. But, calcium (Ca), Copper (Cu) and boron (B) contents were distinctly reduced by salicylic acid treatment, as compared to other treatments. Ethrel and maleic hydrazide also were effective in reducing these contents, but not to the extent of salicylic acid. Calcium and boron being closely related to membrane integrity, and copper being an integral part of certain important enzymes, the reductions in these nutrients by salicylic acid treatment could adversely affect the reproductive growth of rice plant, more particularly the pollens, as the cells might have little resistance to the damage caused by the rise in internal temperature by salicylic acid. Unlike leaves, the panicles exhibited large decreases in all the nutrients including potassium which lent support to the above view. Decreased 'K' content in panicles might be due to sluggish translocation of 'K' ions from leaves to the developing panicles. Harper and Balke (1981) observed limited absorption of 'K' with salicylic acid and ferulic acid treatments in oats. Rerkasem and Jamjod (1997) also observed that male sterility was induced by boron deficiency. That copper and calcium deficiencies also might be associated with male sterility in rice were reported by Agarwala *et al*. (1980), and Tian *et al*. (1998) respectively.

It appeared that the chemicals, used for male sterility, resisted the flow of these nutrients in leaves and panicles by some means.

5.4. Phenols and pollen sterility in TS 29

As salicylic acid has been identified as a promising chemical in inducing almost complete male sterility at a concentration of 800 ppm, several other phenols were also tested in TS 29 along with salicylic acid to know their relative efficacy in inducing sterility in TS 29 line. Under Glass house condition, eight phenols were tested (Fig.17), and based on their performance,salicylic acid, cinnamic acid, catechol and pyrocatechol were tested under field condition for their ability in induction of pollen and spikelet sterilities. Under both the situations, salicylic acid almost produced near complete sterility in pollens and spikelets (Plate 9). Catechol and cinnamic acid were the next best phenols for effecting 80 to 90 per cent sterility. The results clearly indicated the superiority of salicylic acid among phenols for obtaining stable and almost complete male sterility, which might facilitate the use of this chemical advantageously in two line breeding using TGMS system, when the prevailing temperature during panicle development remained below CST. Thangaraj and Lakshmi Praba (2000), and Senthil (2001) also obtained best performance using salicylic acid among the phenols in inducing complete sterility. It appeared that though phenols, in general, could cause inhibitory effects on certain metabolic events, salicylic acid might give CST like effect as a result of its capacity to promote cyanide-resistant respiration as discussed elsewhere. In support of the present observations, Delalonde *et al*. (1996) reported inverse relationship of certain phenols with androgenic capacity. They also revealed that phenols were found to ensure maximum pollen sterility leaving the female organs fertile. Similarly, throughout the present investigation the effect of salicylic acid was mainly on inducting male sterility without affecting female organs. This benefit could be best utilized for emasculating TGMS rice plants as a simple and cheap method.

5.5. Effect of certain chemicals and growth regulators on induction of fertility during sterile phase in TS 29

While finding ways to achieve complete male sterility in TGMS lines by using chemicals, it is equally important to find out an effective chemical for inducting fertility as well, when the plants undergo sterile phase with temperatures above CST. This would help in producing and multiplying parent seeds of TGMS lines under any situations. Even as early as 1981, Foster emphasized that the concept of using chemicals to restore fertility in leading male sterile lines had many advantages as compared with selective sterility approach. Brassinolide (BR), Naphthalene acetic acid (NAA), Indole acetic acid (IAA) and ascorbic acid have been used in the present study, as many reports highlighted their growth promoting effects. Giving two sprays of brassinolide at one ppm concentration during third and fifth stages of panicle development proved effective by increasing pollen fertility above 60 per cent and seed set above 50 per cent under Glass house condition (Plate 9). Promising results in terms of pollen fertility and seed set, obtained under field conditions by using brassinolide, showed the efficacy of this chemical for fertility improvement in TS 29 during sterile phase (Fig.18 and Fig.19). Similar result was also reported by Thirthalingappa *et al*. (1999), who observed increased spikelet and filled spikelets per panicle with higher seed set in CMS lines of rice by using brassinolide. Fuzzi *et al*. (1991) also recorded increased grain weight and percentage of ripened grains in rice plants by the application of BR. These beneficial effects could not have occurred but for the positive manipulation of fertility by brassinolide, as was found in the present study.

5.5.1. Soluble protein in fertility induction

Restoration of fertility to a considerable extent by using growth promoting substances during sterile phase of TGMS lines ought to have definite influence on soluble protein content at the time of crisis. Being a major enzyme in photochemical reaction, RuBPcase occupies a predominant portion of soluble protein content of leaves. Hence, any change in soluble protein content will have an associated influence on RuBPcase content also. The present study revealed a definite improvement in soluble protein content as a result of spraying the growth promoting chemicals namely, BR, NAA, IAA and ascorbic acid on TS 29 line during sterile phase (increase between 26 per cent in BR and 15 per cent in ascorbic acid). Among them, BR at one ppm concentration performed better than other growth promoters. The increase in soluble protein content by BR and other chemicals also manifested concomitant and proportionate increase in pollen fertility in the present study, which indicated the close relationship between pollen fertility and increased soluble protein content. Tarchevsky *et al*. (2001) observed increased synthesis of

polypeptide proteins with exogenous application of phytohormones. Tarraf and Ibrahim (1999) reported increased protein content due to BR application. In this context, Sairam (1994) revealed that BR helped the pollen development by increasing nucleic acids and proteins besides Rubisco activity, which might be due to the involvement of BR in transcription and replication (Kahlem *et al*., 1975).

5.5.2. Carbohydrates in fertility induction

While BR and other growth promoting chemicals exercised favourable influence by increasing the total carbohydrate content of leaves (about 7 to 13 per cent), the same growth promoters considerably reduced the starch content in leaves.. As leaves are the sources for assimilate production, they are not supposed to accumulate starch and take the role of storage organs, once the panicle development has initiated. When the assimilate transport from the source leaf is inefficient, the leaves may start accumulating starch in their cells. Higher starch content in leaves, observed in both CST and salicylic acid treated plants, indicated the poor translocation efficiency with accompanying effect of pollen sterility. In the same vein, BR and other growth promoter sprays, might have enhanced the translocation efficiency and prevented the starch synthesis in the source leaves itself to a considerable extent. The increase in total carbohydrates in the present case could be due to increased production of transportable sugars with the outcome of enhanced source activity facilitated by BR and other chemicals. Arun *et al*. (1999) also observed increased leaf carbohydrate content with BR application.

5.5.3. Proline in fertility induction

Many workers have pointed out that proline accumulation in leaves took place consequent to environmental and edaphic stresses, an intrinsic process to alleviate the stress damage as an osmoprotectant. But, the increase in concentration might be at the cost of certain important aminoacids, thereby affecting the protein synthesis. During sterile phase also, the TGMS line TS 29 experiences unfavourable environment and hence, a distinctly higher content of proline was produced in control plants than in plants treated with growth promoters. About 42 per cent decrease of proline content in BR treated leaves indicated that the metabolic

derangement, caused by stressful environment, was set right by BR paving way for optimum production of amino acids and synthesis of proteins which may facilitate increasing the pollen fertility.

5.5.4. Phenols in fertility induction

Obviously, very large reductions in phenol contents were effected by BR (32 per cent) and other growth promoting substances in the present study. As phenols appeared to be inhibitory to normal biochemical processes, such reductions would have promoted pollen fertility in TS 29. The study highlighted the usefulness of BR and other promoters in mitigating adverse stress effects in the form of reducing the phenols.

5.5.5. Certain enzymes in fertility induction

Increased unoxidised auxin contents observed in TS 29 plants treated with BR, NAA, IAA and ascorbic acid during sterile phase indicated reduced IAA oxidase activity. This increase in unoxidised IAA might favourably influence the pollens by retarding sterility. The effect of the reverse action of leaving behind low unoxidised auxin by CST and salicylic acid treatment ending with pollen sterility has been pointed out previously. Dangar and Basu (1992) also revealed low IAA oxidase activity with auxin application.

The favourable effects of spraying synthetic auxin, namely NAA in enhancing nitrate reductase activity has been reported by Sivakumar *et al*. (2001) in pearl millet. Being a dominant enzyme in nitrogen metabolism, any influence to increase its activity could promote the efficient biochemical events related to amino acid and protein synthesis. About 25 per cent increase in activity was seen in NAA treated plants, but other chemicals including BR have also effected more than 11 per cent increase. This increase in NRase would have favoured the TS 29 plants while experiencing sterile phase during panicle development to produce fertile pollens.

The free radical scavenging enzymes namely catalase, peroxidase and superoxide dismutase (SOD) activities were also promoted by these growth promoters, and their effects were more pronounced in increasing SOD activity. As normal functioning of these enzymes are pre-requisites to make the biochemical stream to function without the interference of free radicals and byproducts of metabolically broken down waste materials, TS 29, under stressful environment, would have benefited with the minimum interference of these harmful free radicals and minimising the pollen damage. Among the growth regulators, IAA treatment favoured catalase activity, while BR and ascorbic acid treatments favouring peroxidase and SOD activities respectively. Raza and Gouri (2000) observed increased catalase activity due to the application of ascorbic acid in rice. That the growth promoting chemicals could be used for enhancing the activities of free radical scavenging such as peroxidase and SOD was also revealed by Dangar and Basu (1992).

5.6. CORRELATION STUDIES

5.6.1. Correlation co-efficients of different physiological and biochemical attributes with pollen sterility, induced by high temperature conditions in TS 29

TS 29 plants were subjected to critical sterility temperature during panicle development under Phytotron condition. Values obtained for various physiological and biochemical attributes, estimated during the course of present investigation, have been subjected to correlation co-efficient analysis, for relating these values with the extent of pollen sterility induced by the critical sterility temperature in different replications, and are furnished in Table 58. Very high and significantly negative relationships were obtained for IAA contents of leaves and panicles with pollen sterility (–0.99 and –0.95 respectively). Catalase activity (–0.86), soluble protein content (–0.85), hill reaction (–0.82), total free amino acids (–0.81), superoxide dismutase activity (–0.80), GDH activity (–0.79) and peroxidase activity (–0.72) are the other attributes which recorded highly significant negative relationship with pollen sterility. Photosynthetic rate also revealed highly negative relationship of –0.72 with pollen sterility. Among the parameters that favoured sterility induction, total phenol content revealed the maximum positive relationship

of 0.98 with pollen sterility. Ethylene release rates, recorded in emerging panicles, also showed significantly very high positive correlation of 0.87 with pollen sterility. The other parameters that showed high positive relationship with pollen sterility were diffusive resistance (0.76), proline content (0.73) and starch content (0.57).

5.6.2. Correlation co-efficients of different biochemical and nutrient constituents of leaves of TS 29 with pollen sterility, induced by salicylic acid treatment

Salicylic acid treatment, given during partial sterility phase, enhanced pollen sterility nearer to 100 per cent. Different biochemical and nutrient constituents, recorded in the present study, were related with pollen sterility values of different replications. The 'r' values are furnished in Table 59. Maximum positive relationship of 0.94 existed between total phenol content of leaves and pollen sterility. The other parameters that revealed high positive relationships with pollen sterility were ascorbic acid oxidase (0.77), proline content (0.69), and IAA oxidase activity (0.68). The parameter which revealed highly significant negative relationship with pollen sterility was superoxide dismutase activity (–0.98) followed by calcium content of leaf (–0.97). The other parameters which were also found to exhibit significantly high negative relationship were catalase activity (–0.91), peroxidase activity (–0.89), boron content (–0.86), copper content (-0.81), total free amino acids (–0.79) and soluble protein content (–0.72).

5.7. MORPHOLOGICAL AND ANATOMICAL CHANGES OF ANTHERS AND POLLENS ASSOCIATED WITH MALE STERILITY

Distinct differences in the morphology of anthers and pollen grains were observed in the present study between fertile and sterile TS 29 plants (Plate 10). Fertile anthers were yellow, larger, plumpy and turned blue when stained with iodine-potassium iodide, whereas, the sterile anthers were small, shrivelled, white coloured and did not take up the stain. Kaul (1988) also reported such morphological changes in anthers of male sterile and fertile lines. Chaudhury *et al*. (1981) and Sun and Zhu.(1995) also reported that the fertile anthers had round, oval and larger

pollen grains, whereas, sterile pollen grains were deformed, sickle shaped and crumbled in appearance.

The microtome sections of male sterile anthers showed persistent tapetum, whereas in male fertile anthers, tapetum degenerated with thickened endothecium and epidermal cells. Similar findings were reported by Senthil (2001). Cheng and Huang (1980) opined that under the influence of fertile gene, the anther wall structure was highly altered. Matsui *et al*. (2001) reported floret fertility in the anthers of rice even at high temperatures because of the well developed cavities and thicker locule walls for dehiscence. Prasad and Rao (1996) also observed differences in the anther sac layers of CGMS lines of rice.

The persistent nondisintegrated tapetum in sterile anthers might have inhibited the transport of nutrients to the developing pollen grains. Whereas, in fertile anthers, the disintegrated tapetum might have permitted the entry of assimilates to the interior of anthers (Wang *et al*., 1992). Reduction in nutrient supply due to abnormal tapetum tissue may be one of the reasons for pollen sterility.

5.8. CERTAIN BIOCHEMICAL MARKERS IDENTIFIED IN TS 29

5.8.1. Electrophoresis of soluble protein

5.8.1.1. Leaf protein

The leaf proteins from sterile and fertile TS 29 were subjected to SDS-PAGE to find out any change in the protein fractions. The electrogram is presented in Plate 11. Feeble expression at 55 kDa level and relatively reduced expression of low molecular weight protein (22 kDa) under sterile condition, as compared to fertile condition were observed. The electrogram indicated that the large sub unit of Rubisco at 55 kDa would have been severely affected under sterile condition compared to fertile one.

Miller (1988) reported a reduction of larger sub unit(LSU) of Rubisco at high temperature. According to Hidema *et al*. (1992) the impaired synthesis of LSU under stress accounted for inhibited synthesis of Rubisco holoenzyme. High temperature stress caused membrane damage, protein denaturation and mutation of DNA (Bowler *et al*., 1992). Enhanced expression of low molecular mass protein (22 kDa) was seen in fertile phase. This was in accordance with the findings of Roystephen and Thangaraj (2000).

5.8.1.2. Anther protein

The SDS-PAGE analysis of anther protein showed distinct quantitative differences between fertile and sterile anthers of TS 29 and CO 46. A 38 kDa protein was specific to the fertile line suggesting a role for this protein for fertility in TGMS system (Plate 11). The same protein was present in small amount in CO 46 also, confirming its association with fertility. A small molecular mass protein of 14 kDa was expressed only in sterile condition, and this might be associated with the expression of male sterility. The presence of 48 kDa protein only in TGMS line and its absence in CO 46 suggested a difference in anther polypeptides of TGMS rice TS29. The findings confirmed the report of Huang *et al*. (1994) that a specific protein is associated with fertility expression in PGMS lines. Roystephen (1998) has reported a specific protein with fertility in anthers of TGMS lines. Huang *et al*. (1996) also observed that specific protein bands were absent in male sterile lines compared to their source lines.

5.8.2. Isozyme analysis

Isozymes have a noteworthy impact on regulating plant growth and development (Li *et al*., 1994). Isozyme patterns were reported to be the better biochemical means to analyse the interactions with higher accuracy (Freeling, 1983). In the present study, isozyme patterns of TS 29 line (fertile and sterile condition) were compared with the normal variety, CO 46. Peroxidase and esterase are the two enzymes which have been extensively employed by research workers for biochemical tagging of genotypes and elucidation of several genetical phenomena at the

biochemical level owing to their presence in most of the organs and easy detection (McMillin, 1983; Hondge, 1986).

5.8.2.1. Peroxidase

Analysis of zymogram for peroxidase revealed that PRX 10 was present in TS 29 under sterility and completely absent in CO 46 and TS 29 under fertile conditions. The activity of PRX 15 was uniformly high in both TS 29 and CO 46, while a uniformly low active isozyme PRX 22 was present in TS 29 (fertile and sterile) and CO 46. PRX 32 was strongly present in the replicants of TS 29 under fertile conditions and variety CO 46, and was weakly displayed in TS 29 under sterile condition. PRX 45, was one isozyme with medium activity in both CO 46 and replicants of TS 29 under fertile condition, while PRX 45 was completely missing in TS 29 under sterility. In the present study, the isozyme namely PRX 10 was more related to TGMS character because, it was exclusively present only in TS 29 line grown under high temperature treatment. The presence of PRX 45 in TS 29 line under fertility and CO 46 indicated its possible relationship with fertility character (Plate 12).

The results of the present study agree with the earlier findings of Wu and Xiao (1993) who reported a variation in peroxidase isozyme banding pattern and its activity in TGMS lines. Lie *et al*. (1993) also observed differences in banding pattern of peroxidase isozyme in SD and LD treated plants. Elsy (1997) reported a similar isozyme banding pattern of peroxidase under high and low temperature with the absence of PRX 23 under high temperature.

5.8.2.2. Esterase isozyme

The isozyme studies of esterase enzyme indicated that EST 20 and EST 30 were present with medium activity and EST 39 was present with low activity in all the lines. But, two esterase isozymes namely EST 67 and EST 70 were present with high activity in CO 46 and fertile TS 29, while these bands were completely absent in sterile TS 29 (Plate 12). The results indicated that the isozyme EST 67 and EST 70 were more related to fertility character. Hu and Wan (1991) and

Li *et al*. (1994) reported the relationship of a specific esterase isozyme with male sterility in rice. Lakshmi Praba (1998) has reported the presence of EST 53, a specific isozyme in sterile TS 29. Similarly Li *et al*. (1995) reported the presence of specific esterase band in TGMS line, Annong IS.

5.8.2.3. Superoxide dismutase

The isoenzymatic pattern of superoxide dismutase revealed five isoforms of SOD (SOD 15, SOD 30, SOD 44, SOD 65 and SOD 80). Fertile TS 29 showed a specific band SOD 44 (Plate 12). The results of the present study was in support of the findings of Zhang *et al*. (1994a) and Roystephen (1998) who observed higher SOD activity under fertile condition compared to sterility in PTGMS and TGMS lines respectively. It could be inferred that, high temperature produced more reactive oxygen species causing denaturation of proteins. This might be the cause for the absence of the particular isozyme under sterile condition.

5.9 CONCLUSION

From the present study, it is established that the critical sterility temperature for achieving complete pollen sterility in TS 29 is 27.5^{o}C and the precise thermosensitive stage for effecting complete pollen sterility is 10 to 25 days before heading. When partial sterility temperature prevails, which is below CST during the thermosensitive stage, salicylic acid spray at 800 ppm is effective and cheaper chemical for obtaining stable male sterility without affecting the female fertility. Regarding induction of fertility during the sterile phase of TS 29, two sprays of Brassinolide at one ppm concentration during third and fifth stages of panicle development proved effective in increasing pollen fertility and seed set percentage. Alterations in various physiological and biochemical parameters, related to fertility and sterility changes in TS 29, were highlighted in this study.

In continuation of the present investigation, certain future thrust areas of research are suggested. Further studies to identify suitable molecular markers in TGMS system may be

initiated. Molecular markers provide an unique opportunity to tag genes for hybrid rice breeding by marker assisted selection of TGMS lines and to facilitate efficient transfer of TGMS gene from one genetic background to another. This apart, hardening of sprouted seeds with suitable chemicals or temperature treatments may be tried, and the attendant changes in isozyme pattern may be studied. Such pre-treated line may end with either fertile or sterile pollens as the case may be.

CHAPTER VI

SUMMARY

An investigation was aimed to characterise the thermosensitive nature of an amenable rice line, TS 29, in terms of physiological and biochemical parameters and to identify the particular sensitive stage of rice phenophase for effective male sterility induction, to fix the critical sterility temperature (CST) and to ponder over the possibility of using chemicals and growth regulators to manipulate fertility/sterility induction. Morphological and anatomical changes in anthers and pollens, under fertile and sterile conditions, were also investigated. Isozyme analysis was carried out to reveal the polymorphism of enzymes in sterile and fertile conditions, and to study its potential for using as biochemical markers to screen TS 29 rice line.

Six different experiments were conducted in the present investigation involving the TGMS line, TS 29 and wherever necessary, a known thermo-insensitive variety, CO 46 has been involved as a check for comparison. The results of various experiments are summarised below:

* The critical sterility temperature for achieving complete pollen sterility in TS 29 was found to be a daily mean temperature of 27.5^{o}C.
* The precise thermosensitive stage for effecting 100 per cent sterility in TS 29 was 10 to 25 days before heading. This period coincides the duration from the origination of secondary branch primordia, when the young panicle is about one mm long, covered with white hairs, till the filling phase of pollen and floret when the panicle reached full length and the colour turned to green.
* Severe reductions in IAA and ABA contents in the leaves and young panicles were observed in TS 29 with sterile pollens under CST treatment as compared to that possessing fertile pollens which might have led to pollen sterility. CO 46 revealed only a negligible reduction of IAA and ABA contents in leaves while the young panicles exhibited a small reduction in IAA content, with a slight increase in ABA content.

* Ethylene release rates (ERR) of emerging panicles of sterile TS 29 increased when subjected to CST over that of fertile plants. The excess ethylene under CST might have inflicted harmful effects leading to pollen sterility.
* Photosynthetic rates declined steeply in sensitive TS 29, compared to CO 46 plants subjected to CST. The reduction in current photosynthesis during the vulnerable panicle development stage might have deprived the developing panicles from getting sufficient nourishment leading to sterility.
* Carbohydrate fractions were found reduced under CST, but starch accumulated as a consequence of impaired translocation and low photosynthetic rates which might have deprived the panicles of getting assimilates leading to sterility.
* Hill reaction rate was severely hampered by CST treatment in TS 29, which might be the major cause for reduced photosynthetic rate. Nucleic acid contents also decreased under CST.
* Reduction in soluble protein content by CST treatment in sensitive TS 29 might have seriously jeopardized RuBP carboxylase activity that would have lowered photosynthetic rate and reduced assimilate supply with an ultimate effect of pollen sterility.
* Proline accumulated several folds in sterile TS 29 under CST treatment. Similarly the total phenols also increased. The accumulation of such secondary metabolites might be at the cost of normal metabolic substrates, which might have caused poor assimilate supply to developing pollens causing sterility.
* Decreased IAA content was found associated with male sterility and the reduction in the functional auxin was due to increased activity of IAA oxidase.
* Nitrate reductase activity reduced in CST induced male sterile TS 29 that would have destabilized the nitrogen assimilation during panicle development resulting in sterile pollens due to poor synthesis of amino acids.
* Ascorbic acid oxidase activity increased in male sterile TS 29 over male fertile plants. The insufficient ascorbic acid content seemed to have lessened antioxidant activity.

- Glutamate dehydrogenase activity was reduced by CST treatment in TS 29, which might have seriously affected the photochemical activities of chloroplasts resulting in pollen sterility.
- Activities of free radical scavenging enzymes namely catalase, peroxidase and superoxide dismutase were reduced in CST treated TS 29 plants. Consequently, the levels of reactive hydroxyl radicals might have increased and caused peroxidation of unsaturated lipids of cell membranes in fragile pollen cells of sensitive TS 29 and damaged the structural integrity leading to pollen sterility.
- The reduction in major nutrients namely, nitrogen and potassium, and micronutrients namely, calcium, copper and boron in sterile TS 29 leaves might be attributed to the susceptibility of TS 29 to elevated temperature. These reduced contents might have affected the synthesis of proteins and various metabolic enzymes.
- Total absence or negligible production of certain free amino acids were observed in the CST treated TS 29 line which indicated derangement of the normal metabolic activity in plant system curtailing protein synthesis. Defective protein synthesis during panicle development stage might have led to ineffective pollens.
- Among the polyamines, increased presence of spermidine favoured production of fertile pollens, while putrescine was found to favour sterile pollens.
- Salicylic acid was found to be the best and cheaper alternative to ethrel and maleic hydrazide in inducing pollen sterility. The effect of salicylic acid is attributed to the increase in body temperature of the plant that showed parallelism to CST treatment in induction of sterility.
- Salicylic acid treatments recorded higher seed set percentages compared to ethrel and maleic hydrazide treatments, it appeared that, ethrel and maleic hydrazide affected the female organs to a certain extent in addition to effecting pollen sterility.
- Salicylic acid treatment increased the starch content with attendant reduction in other carbohydrate fractions. Paucity of transportable assimilates in leaves at the time of reproductive organ development, might be a cause for pollen sterility in TS 29.

* Decrease in soluble protein content and reduction in amino acid pool during flowering by salicylic acid might have affected the CO_2 fixation and assimilate synthesis, as RuBP carboxylase is a major constituent of soluble protein.
* Increment in proline and phenol contents, affected by salicylic acid treatments, might have been due to diversion in normal metabolic process. This diversion might lead to imbalance in enzyme activity and decrease in protein synthesis during the crucial stage of reproductive development.
* The low unoxidised auxin content in leaves of TS 29, treated with salicylic acid indicated that auxin content would have been much reduced by enhanced active salicylic acid treatment, and this reduced auxin level would not have favoured proper pollen development.
* Salicylic acid doubled the activity of ascorbic acid oxidase and thereby, depleted ascorbic acid content leading to deficiency in antioxidant system.
* Activities of the free radical scavenging enzymes namely, catalase, peroxidase and superoxide dismutase were found reduced under salicylic acid treatments favouring the accumulation of free radicals leading to membrane peroxidative damage and pollen sterility.
* Contents of micronutrients, such as, calcium, copper and boron were distinctly reduced by salicylic acid treatment and the deficiencies adversely affected the reproductive growth of rice, more particularly, the pollens.
* Spraying BR, NAA, IAA and ascorbic acid during sterile phase increased the pollen fertility and seed set in TS 29.
* Two sprays of BR at one ppm concentration, during third and fifth stages of panicle development, proved effective in increasing pollen fertility, seed set percentage by certain favourably influencing certain biochemical constituents like soluble protein and carbohydrates. Free radical scavenging enzymes namely catalase, peroxidase and SOD activities were also enhanced by application of BR.
* Light microscope and microtome section studies showed shrivelled, ill-filled and malformed anthers with pollens under high temperature treatment.

* SDS-PAGE analysis showed a reduction in the larger subunit (LSU) of Rubisco under sterility, while isozyme analysis of peroxidase, esterase and superoxide dismutase showed polymorphism with distinct isoform and bands in sterile and fertile conditions. The isoforms showed differences between TS 29 line and CO 46. The specific isozyme bands, present only in TS 29 line, showed the potential of using the isozyme patterns as efficient biochemical marker for screening TS 29.

Table 12. Effect of different temperature regimes on pollen sterility of TS 29

Temperature regimes (^{o}C)	Mean temperature (^{o}C)	Pollen sterility (%)
36.0 / 24.0	30.0	100.0
35.0 / 23.0	29.0	100.0
34.0 / 23.0	28.5	100.0
34.0 / 22.0	28.0	100.0
34.0 / 21.0	27.5	100.0
33.0 / 21.0	27.0	97.5
33.0 / 20.0	26.5	90.3
32.0 / 20.0	26.0	67.0
31.0 / 20.0	25.5	30.6
30.0 / 20.0	25.0	19.3
30.0 / 19.0	24.5	11.6
29.0 / 19.0	24.0	3.5
29.0 / 18.0	23.5	0.8
28.0 / 18.0	23.0	0.6
	CD (P=0.05)	3.79

Table 13. Effect of thermoperiodic treatment during different panicle development stages on pollen sterility in TS 29

Stages	Pollen sterility (%)
0 – 30 DBH	100.00
0 – 20 DBH	93.75
0 – 10 DBH	43.00
5 – 30 DBH	93.75
5 – 25 DBH	100.00
5 – 10 DBH	26.75
10 – 30 DBH	95.50
10 – 25 DBH	100.00
10 – 20 DBH	92.50
15 – 30 DBH	64.00
15 – 20 DBH	66.00
20 – 30 DBH	48.00
CD (P=0.05)	0.577

DBH : Days before heading

Table 15. Ethylene release rate (ERR) from panicles of TS 29 and CO 46

Treatments	Ethylene concentration (µ moles min^{-1})	Area ($mV\ s^{-1}$)	Height of the peak (mm)
TS 29 – Glass house (Fertile pollen)	2.403 (100.0)	2.014 (100.0)	0.124 (100.0)
TS 29 – Phytotron (Sterile pollen)	5.621 (234.0)	5.321 (264.2)	0.532 (429.0)
CO 46 – Glass house	1.999 (100.0)	1.724 (100.0)	0.092 (100.0)
CO 46 – Phytotron	2.011 (101.0)	1.921 (111.4)	0.101 (110.0)
Mean	3.001	2.741	0.212
CD (P=0.05)	0.033	1.857	0.004

(Values in parentheses are per cent change in Phytotron plants as compared to Glass house plants)

Table 23. Contents of certain free amino acids in leaves of TS 29 (Paper chromatography) at stage VII of panicle development

Free amino acids	Fertile lines (mg 100 g^{-1})	Sterile lines (mg 100 g^{-1})
Leucine	0.139	0.009
Iso-leucine	0.290	ND
Lysine	0.164	ND
Cysteine	0.224	0.132
Methionine	0.137	0.120
Tyrosine	0.277	ND
Histidine	0.181	0.098

ND – Not detectable

Table 24. HPLC profile of phenolic compounds in leaves of TS 29 at stage VII of panicle development

Treatments	Phenolic compounds (mg g^{-1})					
	Salicylic acid	Ferulic acid	Benzoic acid	Catechol	Pyro-catechol	Syringic acid
Leaves of sterile plant	5.34	ND	ND	1.73	3.61	ND
Leaves of fertile plant	ND	ND	ND	ND	ND	ND

ND – Not detectable

Table 25. HPLC profile of polyamine contents in leaves and young panicles of TS 29 at stage VII of panicle development

Treatments	Polyamines (μ mol g^{-1})		
	Spermidine	Putrescine	Cadavarine
Leaves of fertile plant	2.13	ND	0.39
Leaves of sterile plant	ND	3.84	0.33
Panicles of fertile plant	0.05	ND	ND
Panicles of sterile plant	ND	0.03	ND

ND-Not detectable

Table 26. Effect of spraying certain chemicals and growth regulators during panicle development on pollen sterility (%) of TS 29

Treatments	Pollen sterility (%)		
	Season I	Season II	Pooled mean
Control	57.22	17.04	37.13
Salicylic acid (800 ppm)	99.60	98.13	98.87
Salicylic acid (1000 ppm)	99.95	98.78	99.36
Ethrel (800 ppm)	75.88	88.50	82.19
Maleic hydrazide (2000 ppm)	81.91	80.71	81.31
Mean	82.91	76.63	
CD (P=0.05)	1.698	2.498	
For Pooled Analysis CD (P=0.05)	S	0.804	
	T	1.431	
	S x T	2.023	

Table 27. Effect of spraying certain chemicals and growth regulators during panicle development on crossed seed set (%) of TS 29

Treatments	Crossed seed set percentage					
	Rope pulling method			Clipping method		
	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean
Control	-	-	-	-	-	-
Salicylic acid (800 ppm)	26.60	25.90	26.25	39.55	37.86	38.70
Salicylic acid (1000 ppm)	25.88	24.77	25.32	39.34	37.08	38.20
Ethrel (800 ppm)	18.96	20.66	19.81	26.28	30.08	28.18
Maleic hydrazide (2000 ppm)	23.61	21.40	22.54	31.36	31.23	31.30
Mean	19.01	18.56		27.31	27.25	
CD (P=0.05)	0.772	0.659		0.884	1.423	
For Pooled Analysis CD (P=0.05)	S	0.271		S	0.703	
	T	0.481		T	0.794	
	S x T	0.680		S x T	1.122	

Table 33. Effect of sterility inducing chemicals and growth regulators on contents of individual phenols (mg g^{-1}) in leaves of TS 29 at panicle development and flowering stages

Treatments	Phenolic content (mg g^{-1})						
	Salicylic acid	Ferulic acid	Benzoic acid	Catechol	Pyrocate-chol	Syringic acid	Area covered (mv s^{-1})
Control	-	-	-	-	-	1.601	169135
Salicylic acid (800 ppm)	6.321	-	5.553	1.104	-	-	756969 847658 207694
Salicylic acid (1000 ppm)	7.728	1.057	3.751	1.673	3.655	-	865556 738941 168729 314609 624120
Ethrel (800 ppm)	1.053	1.112	-	0.052	-	-	126082 177467 9853
Maleic hydrazide (2000 ppm)	-	2.199	-	2.674	-	-	350843 502888

Data not analysed statistically

Table 35. Effect of sterility inducing chemicals and growth regulators on ethylene release rate (µ moles min^{-1}) from emerging panicles of TS29

Treatments	Height of the peak (mm)	Area ($mV\ s^{-1}$)	C_2H_4 conc. (µ moles min^{-1})
Control	0.106	1.630	1.906
Salicylic acid (800 ppm)	0.146	5.921	4.765
Salicylic acid (1000 ppm)	0.178	6.032	5.989
Ethrel (800 ppm)	0.123	3.999	4.808
Maleic hydrazide (2000 ppm)	0.127	3.017	4.031
	CD (P=0.05)		0.046

Table 44. Effect of spraying different phenols on percentages of pollen and spikelet sterility in TS 29 under Glass house condition

Phenols (1000ppm)	Pollen sterility (%)	Spikelet sterility (%)
Control	13.31	16.51
Salicylic acid	98.00	99.50
Ferulic acid	52.24	60.52
Benzoic acid	63.72	70.21
Chlorogenic acid	75.57	80.25
Cinnamic acid	85.62	88.75
Syringic acid	50.25	59.30
Catechol	90.50	92.50
Pyrocatechol	80.53	82.25
Mean	67.719	72.20
CD (P=0.05)	0.237	0.231

Table 45. Effect of spraying certain phenols on the induction of male sterility in TS 29 under field condition

Phenols (1000 ppm)	Pollen sterility (%)	Spikelet sterility (%)
Control	15.8	16.5
Salicylic acid	97.5	98.5
Cinnamic acid	80.3	85.5
Catechol	88.5	90.1
Pyrocatechol	65.5	70.0
Mean	62.5	62.7
CD (P=0.05)	0.44	0.44

Table 46. Effect of certain chemicals and growth regulators on induction of fertility during sterile phase in TS 29 under Glass house condition

Treatments	Pollen fertility (%)	Seed set (%)
Control	5.4	0.0
BR (1 ppm)	63.3	51.4
NAA (50 ppm)	40.5	32.1
IAA (50 ppm)	56.4	40.6
Ascorbic acid (100 ppm)	25.4	21.2
Mean	40.1	29.4
CD (P=0.05)	0.64	0.45

Table 47. Effect of spraying certain chemicals and growth regulators during panicle development on pollen fertility (%) and selfed seed set (%) of TS 29 under field condition

Treatments	Pollen fertility (%)	Seed set (%)
Control	2.95	0.00
BR (1 ppm)	40.21	36.72
NAA (50 ppm)	21.40	16.38
IAA (50 ppm)	30.26	28.39
Ascorbic acid (100 ppm)	19.80	11.15
Mean	23.09	18.56
CD (P=0.05)	0.589	0.245

Table 48. Effect of fertility inducing chemicals and growth regulators on soluble protein content (mg g^{-1}) of TS 29 leaves at panicle development and flowering stages

Treatments	Panicle development		Flowering	Mean
	Five days after first treatment (Stage III)	Five days after second treatment (Stage V)		
Control	7.56	11.55	12.84	10.65 (100)
BR (1 ppm)	11.14	14.24	14.95	13.44 (126)
NAA (50 ppm)	10.04	13.94	14.25	12.74 (120)
IAA (50 ppm)	10.14	13.73	14.95	12.94 (122)
Ascorbic acid (100 ppm)	9.95	12.83	13.94	12.24 (115)
Mean	9.77	13.26	14.19	12.40
CD (P=0.05)	0.0481	0.104	0.200	

(Values in parenthesis are per cent change over control)

Table 49. Effect of fertility inducing chemicals and growth regulators on starch content (mg g^{-1}) of TS 29 leaves at panicle development and flowering stages

Treatments	Panicle development		Flowering	Mean
	Five days after first treatment (Stage III)	Five days after second treatment (Stage V)		
Control	35.94	39.93	40.05	38.64 (100)
BR (1 ppm)	28.76	31.16	36.74	32.22 (83)
NAA (50 ppm)	27.79	30.78	38.58	32.38 (84)
IAA (50 ppm)	28.63	30.95	31.76	30.45 (79)
Ascorbic acid (100 ppm)	29.90	35.27	40.06	35.08 (91)
Mean	30.20	33.62	37.44	33.75
CD (P=0.05)	0.150	0.144	0.396	

(Values in parenthesis are per cent change over control)

Table 50. Effect of fertility inducing chemicals and growth regulators on total carbohydrates content (mg g^{-1}) of TS 29 leaves at panicle development and flowering stages

Treatments	Panicle development		Flowering	Mean	
	Five days after first treatment (Stage III)	Five days after second treatment (Stage V)			
Control	81.34	88.84	80.15	83.44	(100)
BR (1 ppm)	85.53	90.34	98.14	91.34	(109)
NAA (50 ppm)	88.15	95.44	98.24	93.94	(113)
IAA (50 ppm)	85.25	98.84	99.16	94.42	(113)
Ascorbic acid (100 ppm)	83.24	90.04	94.15	89.14	(107)
Mean	84.70	92.70	93.97	90.46	
CD (P=0.05)	0.109	0.371	0.504		

(Values in parenthesis are per cent change over control)

Table 51. Effect of fertility inducing chemicals and growth regulators on proline content (mg g^{-1}) of TS 29 leaves at panicle development and flowering stages

Treatments	Panicle development		Flowering	Mean	
	Five days after first treatment (Stage III)	Five days after second treatment (Stage V)			
Control	1.85	2.14	3.16	2.38	(100)
BR (1 ppm)	0.94	1.15	2.05	1.38	(58)
NAA (50 ppm)	1.05	1.65	2.75	1.82	(76)
IAA (50 ppm)	1.08	1.44	2.24	1.59	(67)
Ascorbic acid (100 ppm)	1.25	1.74	2.75	1.91	(80)
Mean	1.23	1.63	2.59	1.82	
CD (P=0.05)	0.022	0.027	0.064		

(Values in parenthesis are per cent change over control)

Table 52. Effect of fertility inducing chemicals and growth regulators on total phenols (mg g^{-1}) of TS 29 leaves at panicle development and flowering stages

Treatments	Panicle development		Flowering	Mean	
	Five days after first treatment (Stage III)	Five days after second treatment (Stage V)			
Control	2.45	3.95	4.15	3.52	(100)
BR (1 ppm)	2.04	2.55	2.65	2.41	(68)
NAA (50 ppm)	2.15	2.75	2.83	2.58	(73)
IAA (50 ppm)	2.25	2.45	2.74	2.48	(70)
Ascorbic acid (100 ppm)	2.15	2.76	2.84	2.58	(73)
Mean	2.21	2.89	3.04	2.71	
CD (P=0.05)	0.024	0.023	0.052		

(Values in parenthesis are per cent change over control)

Table 53. Effect of fertility inducing chemicals and growth regulators on IAA oxidase activity (µ moles of unoxidised auxin g^{-1} hr^{-1}) of TS 29 leaves at panicle development and flowering stages

Treatments	Panicle development		Flowering	Mean
	Five days after first treatment (Stage III)	Five days after second treatment (Stage V)		
Control	16.10	16.17	16.37	16.20 (100)
BR (1 ppm)	16.27	16.50	16.92	16.56 (102)
NAA (50 ppm)	15.70	16.55	17.45	16.57 (102)
IAA (50 ppm)	16.69	16.77	17.25	16.90 (104)
Ascorbic acid (100 ppm)	16.00	16.25	16.99	16.41 (101)
Mean	16.15	16.45	16.99	16.53
CD (P=0.05)	0.063	0.030	0.019	

(Values in parenthesis are per cent change over control)

Table 54. Effect of fertility inducing chemicals and growth regulators on nitrate reductase activity (µ moles NO_2 g^{-1} hr^{-1}) of TS 29 leaves at panicle development and flowering stages

Treatments	Panicle development		Flowering	Mean
	Five days after first treatment (Stage III)	Five days after second treatment (Stage V)		
Control	0.314	0.545	0.345	0.401 (100)
BR (1 ppm)	0.324	0.593	0.435	0.451 (112)
NAA (50 ppm)	0.395	0.644	0.495	0.511 (127)
IAA (50 ppm)	0.364	0.614	0.452	0.477 (119)
Ascorbic acid (100 ppm)	0.344	0.564	0.431	0.446 (111)
Mean	0.348	0.592	0.432	0.457
CD (P=0.05)	0.004	0.003	0.003	

(Values in parenthesis are per cent change over control)

Table 55. Effect of fertility inducing chemicals and growth regulators on catalase activity (Enzyme units x 10^4 g^{-1} min^{-1}) of TS 29 leaves at panicle development and flowering stages

Treatments	Panicle development		Flowering	Mean	
	Five days after first treatment (Stage III)	Five days after second treatment (Stage V)			
Control	2.64	3.83	3.98	3.48	(100)
BR (1 ppm)	2.91	3.96	4.13	3.66	(105)
NAA (50 ppm)	2.93	3.62	4.03	3.53	(101)
IAA (50 ppm)	2.98	4.01	4.24	3.74	(107)
Ascorbic acid (100 ppm)	2.77	3.86	3.94	3.52	(101)
Mean	2.85	3.86	4.06	3.59	
CD (P=0.05)	0.023	0.024	0.025		

(Values in parenthesis are per cent change over control)

Table 56. Effect of fertility inducing chemicals and growth regulators on peroxidase activity (Enzyme units l^{-1} hr^{-1}) of TS 29 leaves at panicle development and flowering stages

Treatments	Panicle development		Flowering	Mean
	Five days after first treatment (Stage III)	Five days after second treatment (Stage V)		
Control	176.50	182.43	185.43	181.45 (100)
BR (1 ppm)	182.43	188.35	193.33	188.04 (104)
NAA (50 ppm)	177.55	183.35	189.35	183.42 (101)
IAA (50 ppm)	180.60	184.43	191.25	185.43 (102)
Ascorbic acid (100 ppm)	179.30	182.95	188.40	183.55 (101)
Mean	179.28	184.30	189.55	184.38
CD (P=0.05)	1.526	0.421	0.194	

(Values in parenthesis are per cent change over control)

Table 57. Effect of fertility inducing chemicals and growth regulators on SOD activity (Enzyme units mg^{-1} protein hr^{-1}) of TS 29 leaves at panicle development and flowering stages

Treatments	Panicle development		Flowering	Mean	
	Five days after first treatment (Stage III)	Five days after second treatment (Stage V)			
Control	7.17	6.95	6.04	6.72	(100)
BR (1 ppm)	8.52	8.64	8.36	8.51	(127)
NAA (50 ppm)	8.24	8.32	8.33	8.29	(123)
IAA (50 ppm)	8.12	8.24	8.26	8.21	(122)
Ascorbic acid (100 ppm)	8.64	8.75	8.56	8.65	(129)
Mean	8.14	8.18	7.91	8.08	
CD (P=0.05)	0.024	0.020	0.025		

(Values in parenthesis are per cent change over control)

Table 58. Correlation co-efficients of different physiological and biochemical attributes with pollen sterility induced by high temperature condition in TS 29

Sl.No.	Variables	'r' value
1.	**IAA (leaves)**	-0.99**
2.	IAA (panicles)	-0.95**
3.	ERR (panicles)	0.87**
4.	Photosynthetic rate	-0.72**
5.	Transpiration rate	-0.61*
6.	Diffusive resistance	0.76**
7.	Hill reaction	-0.82**
8.	Soluble protein	-0.85**
9.	RNA	-0.49*
10.	DNA	-0.42*
11.	Starch	0.57**
12.	Total carbohydrate	-0.48*
13.	Proline	0.73**
14.	Total phenol content	0.98**
15.	Total free aminacids	-0.81**
16.	Ascorbic acid	-0.64*
17.	IAA oxidase activity	0.36*
18.	NRase activity	-0.68*
19.	Ascorbic acid oxidase	0.72**
20	GDH activity	-0.79**
21.	Catalase activity	-0.86**
22.	Peroxidase activity	-0.72**
23.	SOD activity	-0.80**
24.	Sterility (%)	1.00

Table 59. Correlation co-efficients of different biochemical and nutrient constituents of leaves with pollen sterility induced by salicylic acid treatment during stages III and V of panicle development in TS 29

Sl.No.	Variables	'r' value
1.	**Soluble protein**	-0.72**
2.	Starch	0.49*
3.	Total carbohydrate	-0.33*
4.	Total phenol	0.94**
5.	Proline	0.69**
6.	Total free amino acid	-0.79**
7.	NRase activity	-0.64*
8.	IAA oxidase activity	0.68**
9.	Catalase activity	-0.91**
10.	Peroxidase activity	-0.89**
11.	SOD activity	-0.98**
12.	Ascorbic acid oxidase activity	0.77**
13.	Potassium content	-0.67**
14.	Calcium content	-0.97**
15.	Copper content	-0.81**
16.	Boron content	-0.86**
17.	Sterility	1.00

Table 14. IAA and ABA contents of leaves and emerging panicles of fertile and sterile TS 29 and CO 46

Treatments	IAA content (p moles g^{-1} FW)		ABA content (n moles g^{-1} FW)	
	Leaves	Young panicles	Leaves	Young panicles
TS 29 – Glass house (Fertile pollen)	745.95 (100.0)	650.10 (100.0)	0.584 (100.0)	0.401 (100.0)
TS 29 – Phytotron (Sterile pollen)	620.31 (83.2)	591.03 (90.9)	0.157 (26.9)	0.184 (45.9)
CO 46 – Glass house	1016.09 (100.0)	723.21 (100.0)	1.865 (100.0)	0.245 (100.0)
CO 46 – Phytotron	992.37 (97.6)	721.95 (99.8)	1.721 (92.3)	0.251 (102.4)
Mean	843.68	671.57	1.082	0.270
CD (P=0.05)	1.983	2.958	0.096	0.021

(Values in parentheses are per cent change in Phytotron plants as compared to Glass house plants)

Table 16. Gas exchange parameters in leaves of TS 29 and CO 46 at stage VII of panicle development

Treatments	Photosynthetic rate (P_n) (μ mol CO_2 m^{-2} s^{-1})	Transpiration rate (E) (m mol H_2O m^{-2} s^{-1})	Diffusive resistance (r_s) (s cm^{-1})
TS 29 – Glass house (Fertile pollen)	20.50 (100.0)	7.50 (100.0)	5.60 (100.0)
TS 29 – Phytotron (Sterile pollen)	13.10 (63.9)	7.00 (93.3)	6.49 (114.3)
CO 46 – Glass house	22.50 (100.0)	7.70 (100.0)	5.20 (100.0)
CO 46 – Phytotron	20.16 (89.6)	7.50 (97.4)	5.40 (103.9)
Mean	19.07	7.43	5.65
CD (P=0.05)	0.377	0.262	0.231

(Values in parentheses are per cent change in Phytotron plants as compared to Glass house plants)

Table 17. Carbohydrate fractions in leaves of TS 29 and CO 46 at stage VII of panicle development

Treatments	Reducing sugars (mg g^{-1})		Total sugars (mg g^{-1})		Starch content (mg g^{-1})		Total carbohydrates (mg g^{-1})	
TS 29 – Glass house (Fertile pollen)	7.52	(100.0)	24.29	(100.0)	37.14	(100.0)	77.91	(100.0)
TS 29 – Phytotron (Sterile pollen)	5.61	(74.6)	16.93	(69.7)	51.26	(138.0)	65.43	(83.9)
CO 46 – Glass house	7.43	(100.0)	24.71	(100.0)	34.32	(100.0)	85.39	(100.0)
CO 46 – Phytotron	7.34	(98.7)	23.21	(93.9)	33.98	(99.0)	84.65	(99.1)
Mean	6.98		22.29		39.18		78.35	
CD (P=0.05)	0.248		2.069		0.967		0.545	

(Values in parentheses are per cent change in Phytotron plants as compared to Glass house plants)

Table 18. Changes in hill reaction rate and nucleic acid contents in leaves of TS 29 and CO 46 at stage VII of panicle development

Treatments	Hill reaction rate (µ moles DCIP reduced mg^{-1} chlorophyll hr^{-1})	RNA content (µg g^{-1})	DNA content (µg g^{-1})
TS 29 – Glass house (Fertile pollen)	39.68 (100.0)	563.0 (100.0)	336.0 (100.0)
TS 29 – Phytotron (Sterile pollen)	27.41 (69.1)	391.0 (69.5)	210.0 (62.5)
CO 46 – Glass house	41.30 (100.0)	580.0 (100.0)	365.0 (100.0)
CO 46 – Phytotron	40.96 (99.2)	578.0 (99.6)	360.0 (98.6)
Mean	37.34	528.0	317.8
CD (P=0.05)	0.793	2.15	3.01

(Values in parentheses are per cent change in Phytotron plants as compared to Glass house plants)

Table 19. Certain biochemical constituents in leaves of TS 29 and CO 46 at stage VII of panicle development

Treatments	Soluble protein (mg g^{-1})		Proline content (mg g^{-1})		Total phenols (mg g^{-1})		Total free amino acid (mg g^{-1})		Ascorbic acid content (mg g^{-1})	
TS 29 – Glass house (Fertile pollen)	7.66	(100.0)	1.09	(100.0)	2.91	(100.0)	2.99	(100.0)	56.83	(100.0)
TS 29 – Phytotron (Sterile pollen)	4.62	(60.3)	2.34	(214.7)	4.12	(141.6)	1.35	(45.0)	36.64	(64.5)
CO 46 – Glass house	8.21	(100.0)	0.98	(100.0)	2.55	(100.0)	2.85	(100.0)	70.96	(100.0)
CO 46 – Phytotron	8.17	(99.5)	1.35	(137.8)	2.62	(102.5)	2.77	(97.2)	69.45	(97.9)
Mean	7.17		1.44		3.05		2.24		58.47	
CD (P=0.05)	0.197		0.089		0.132		0.034		0.388	

(Values in parentheses are per cent change in Phytotron plants as compared to Glass house plants)

Table 20. Variations in certain enzyme activities in leaves of TS 29 and CO 46 at stage VII of panicle development

Treatments	IAA oxidase activity (µ moles of unoxidised auxin g^{-1} hr^{-1})		NR activity (µ moles NO_2 g^{-1} hr^{-1})		AAO activity (Enzyme units g^{-1} min^{-1})		GDH activity (n moles of NADH oxidised mg^{-1} protein min^{-1})	
TS 29 – Glass house (Fertile pollen)	15.62	(100.0)	0.582	(100.0)	3.33	(100.0)	2.76	(100.0)
TS 29 – Phytotron (Sterile pollen)	9.31	(59.6)	0.341	(58.6)	8.42	(252.8)	0.92	(33.3)
CO 46 – Glass house	15.33	(100.0)	0.645	(100.0)	2.12	(100.0)	2.51	(100.0)
CO 46 – Phytotron	14.94	(97.5)	0.640	(99.2)	2.99	(141.0)	1.99	(79.3)
Mean	13.80		0.552		4.22		2.05	
CD (P=0.05)	0.036		0.003		0.049		0.052	

(Values in parentheses are per cent change in Phytotron plants as compared to Glass house plants)

Table 21. Activities of active oxygen species scavenging enzymes in leaves of TS 29 and CO 46 at stage VII of panicle development

Treatments	Catalase (Enzyme units × 10^4 g^{-1} min^{-1})	Peroxidase (Enzyme units l^{-1} hr^{-1})	Superoxide dismutase (Enzyme mg^{-1} protein min^{-1})
TS 29 – Glass house (Fertile pollen)	2.98 (100.0)	175.00 (100.0)	8.62 (100.0)
TS 29 – Phytotron (Sterile pollen)	1.45 (48.7)	129.00 (73.7)	4.11 (47.7)
CO 46 – Glass house	3.92 (100.0)	138.00 (100.0)	9.16 (100.0)
CO 46 – Phytotron	3.81 (97.2)	129.00 (93.5)	8.77 (95.7)
Mean	3.04	142.75	7.67
CD (P=0.05)	0.086	2.141	0.123

(Values in parentheses are per cent change in Phytotron plants as compared to Glass house plants)

Table 22. Nutrient status of leaves of TS 29 and CO 46 at stage VII of panicle development

Treatments	Nitrogen (%)		Potassium (%)		Calcium (µg g^{-1})		Copper (µg g^{-1})		Boron (µg g^{-1})	
TS 29 – Glass house (Fertile pollen)	2.99	(100.0)	1.52	(100.0)	203.00	(100.0)	106.00	(100.0)	39.00	(100.0)
TS 29 – Phytotron (Sterile pollen)	2.49	(83.0)	1.03	(67.8)	155.00	(76.4)	82.00	(77.4)	22.00	(56.4)
CO 46 – Glass house	3.01	(100.0)	1.49	(100.0)	208.00	(100.0)	120.00	(100.0)	37.00	(100.0)
CO 46 – Phytotron	2.94	(97.7)	1.40	(94.0)	205.00	(98.9)	110.00	(91.7)	35.00	(94.6)
Mean	2.86		1.36		192.75		104.50		33.25	
CD (P=0.05)	0.147		0.058		2.050		1.778		2.376	

(Values in parentheses are per cent change in Phytotron plants as compared to Glass house plants)

Table 28. Effect of sterility inducing chemicals and growth regulators on starch content (mg g^{-1}) in leaves of TS 29 at panicle development
and flowering stages

Treatments	Panicle development						Flowering		
	Five days after first treatment (Stage III)			Five days after second treatment (Stage V)					
	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean
Control	30.23	28.85	29.54	34.43	34.16	34.29	38.18	34.32	36.25
Salicylic acid (800 ppm)	32.22	26.64	29.43	42.23	40.33	41.28	50.18	48.19	49.18
Salicylic acid (1000 ppm)	34.44	26.84	30.64	44.46	41.33	42.89	52.24	45.55	48.90
Ethrel (800 ppm)	30.14	27.07	28.60	39.23	42.25	40.74	40.25	43.38	41.81
Maleic hydrazide (2000 ppm)	29.93	28.83	29.38	38.27	40.17	39.22	40.21	10.28	25.24
Mean	31.39	27.65		39.73	39.65		44.21	36.34	
CD (P=0.05)	0.020	0.043		0.056	0.068		0.042	14.172	
For pooled analysis CD (P=0.05)	S	0.017		S	0.032		S	5.029	
	T	0.022		T	0.042		T	6.712	
	S x T	0.032		S x T	0.059		S x T	9.492	

Table 29. Effect of sterility inducing chemicals and growth regulators on total carbohydrate content (mg g^{-1}) in leaves of TS 29 at panicle development and flowering stages

Treatments	Panicle development						Flowering		
	Five days after first treatment (Stage III)			Five days after second treatment (Stage V)					
	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean
Control	69.91	72.24	71.08	84.43	85.35	84.89	86.63	87.85	87.24
Salicylic acid (800 ppm)	60.24	67.05	63.64	70.18	80.06	75.12	77.35	80.14	78.74
Salicylic acid (1000 ppm)	59.94	68.84	64.39	68.64	81.15	74.90	75.41	80.04	77.73
Ethrel (800 ppm)	60.95	70.59	65.77	55.81	83.33	69.57	84.31	83.34	83.83
Maleic hydrazide (2000 ppm)	69.91	70.03	69.97	75.88	80.51	78.19	85.02	84.45	84.73
Mean	64.19	69.75		72.99	84.08		83.74	85.16	
CD (P=0.05)	0.008	0.413		NS	0.161		0.006	0.019	
For pooled analysis CD (P=0.05)	S	0.157		S	9.09		S	0.005	
	T	0.195		T	12.146		T	0.009	
	S x T	0.276		S x T	NS		S x T	0.014	

Table 30. Effect of sterility inducing chemicals and growth regulators on soluble protein content (mg g^{-1}) in leaves of TS 29 at panicle development and flowering stages

Treatments	Panicle development						Flowering		
	Five days after first treatment (Stage III)			Five days after second treatment (Stage V)					
	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean
Control	7.33	6.91	7.12	12.62	10.24	11.43	12.93	12.24	12.59
Salicylic acid (800 ppm)	6.64	6.24	6.44	11.04	8.37	9.70	11.33	10.23	10.78
Salicylic acid (1000 ppm)	6.76	6.11	6.43	11.25	8.32	9.79	11.43	10.41	10.92
Ethrel (800 ppm)	6.75	6.93	6.84	11.95	9.71	10.83	11.42	11.21	11.31
Maleic hydrazide (2000 ppm)	6.77	6.89	6.83	12.05	9.84	10.95	11.95	11.23	11.59
Mean	6.85	6.44		11.79	9.29		11.82	11.07	
CD (P=0.05)	0.002	0.002		0.030	0.004		0.009	0.012	
For pooled analysis CD (P=0.05)	S	0.002		S	0.014		S	0.005	
	T	0.001		T	0.014		T	0.007	
	S x T	0.002		S x T	0.020		S x T	0.010	

Table 31. Effect of sterility inducing chemicals and growth regulators on proline content (mg g^{-1}) in leaves of TS 29 at panicle development
and flowering stages

Treatments	Panicle development						Flowering		
	Five days after first treatment (Stage III)			Five days after second treatment (Stage V)					
	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean
Control	0.84	0.98	0.91	1.42	1.54	1.48	1.53	1.92	1.73
Salicylic acid (800 ppm)	0.93	1.12	1.02	4.64	4.39	4.52	4.06	4.96	4.51
Salicylic acid (1000 ppm)	0.95	1.22	1.08	4.25	5.02	4.64	4.03	5.35	4.69
Ethrel (800 ppm)	0.84	1.01	0.92	3.15	3.16	3.16	3.03	3.74	3.38
Maleic hydrazide (2000 ppm)	0.83	1.02	0.93	3.93	3.23	3.58	3.74	3.82	3.78
Mean	0.88	1.07		3.48	3.47		3.28	3.96	
CD (P=0.05)	0.004	0.002		0.004	0.019		0.008	0.004	
For pooled analysis CD (P=0.05)	S	0.001		S	0.006		S	0.003	
	T	0.002		T	0.009		T	0.004	
	S x T	0.003		S x T	0.013		S x T	0.006	

Table 32. Effect of sterility inducing chemicals and growth regulators on total phenols (mg g^{-1}) in leaves of TS 29 at panicle development
and flowering stages

Treatments	Panicle development						Flowering		
	Five days after first treatment (Stage III)			Five days after second treatment (Stage V)					
	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean
Control	1.73	1.74	1.74	2.54	2.36	2.45	3.14	3.22	3.18
Salicylic acid (800 ppm)	2.85	2.93	2.89	3.64	4.32	3.98	5.63	6.66	6.15
Salicylic acid (1000 ppm)	2.73	2.98	2.86	3.61	4.44	4.02	5.57	5.98	5.78
Ethrel (800 ppm)	1.94	1.98	1.96	3.44	2.95	3.19	4.03	3.99	4.01
Maleic hydrazide (2000 ppm)	2.04	2.04	2.04	2.95	2.16	2.56	4.25	3.84	4.05
Mean	2.26	2.33		3.24	3.25		4.53	4.74	
CD (P=0.05)	0.022	0.017		0.051	0.022		0.019	0.005	
For pooled analysis CD (P=0.05)	S	0.012		S	NS		S	0.006	
	T	0.013		T	0.026		T	0.009	
	S x T	0.018		S x T	2.064		S x T	0.013	

Table 34. Effect of sterility inducing chemicals and growth regulators on total free amino acid content (mg g^{-1}) in leaves of TS 29 at panicle
development and flowering stages

Treatments	Panicle development						Flowering		
	Five days after first treatment (Stage III)			Five days after second treatment (Stage V)					
	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean
Control	2.24	2.32	2.28	2.51	2.24	2.37	2.73	2.34	2.53
Salicylic acid (800 ppm)	2.08	2.36	2.22	1.85	2.33	2.09	1.65	2.43	2.04
Salicylic acid (1000 ppm)	2.11	2.33	2.22	1.57	2.03	1.80	1.54	2.14	1.84
Ethrel (800 ppm)	2.05	2.15	2.10	1.74	2.03	1.88	1.65	2.12	1.89
Maleic hydrazide (2000 ppm)	2.09	2.15	2.12	1.78	2.07	1.93	1.86	2.15	2.00
Mean	2.11	2.26		1.89	2.14		1.89	2.23	
CD (P=0.05)	0.092	0.033		0.054	0.032		0.047	0.042	
For pooled analysis CD (P=0.05)	S	0.052		S	0.026		S	0.032	
	T	0.046		T	0.030		T	0.029	
	S x T	0.065		S x T	0.042		S x T	0.042	

Table 36. Effect of sterility inducing chemicals and growth regulators on IAA oxidase activity (µ moles of unoxidised auxin g^{-1} hr^{-1}) in leaves of TS 29 at panicle development and flowering stages

Treatments	Panicle development						Flowering		
	Five days after first treatment (Stage III)			Five days after second treatment (Stage V)					
	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean
Control	15.74	15.25	15.50	16.23	16.22	16.23	17.23	16.02	16.63
Salicylic acid (800 ppm)	15.43	15.33	15.38	16.21	15.95	16.08	11.45	11.26	11.36
Salicylic acid (1000 ppm)	15.36	15.31	15.33	16.16	16.02	16.09	12.94	11.37	12.15
Ethrel (800 ppm)	15.42	15.77	15.59	15.02	16.63	15.83	11.14	13.33	12.24
Maleic hydrazide (2000 ppm)	15.54	15.72	15.63	15.24	16.55	15.89	12.04	14.22	13.13
Mean	15.50	15.48		15.77	16.27		12.96	13.24	
CD (P=0.05)	0.042	0.011		0.096	0.029		0.019	0.017	
For pooled analysis CD (P=0.05)	S	0.010		S	0.042		S	0.010	
	T	0.020		T	0.047		T	0.012	
	S x T	0.029		S x T	0.067		S x T	0.017	

Table 37. Effect of sterility inducing chemicals and growth on nitrate reductase activity (µ moles NO_2 g^{-1} hr^{-1}) in leaves of TS 29 at panicle development and flowering stages

Treatments	Panicle development						Flowering		
	Five days after first treatment (Stage III)			Five days after second treatment (Stage V)					
	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean
Control	0.403	0.422	0.412	0.404	0.583	0.493	0.424	0.625	0.524
Salicylic acid (800 ppm)	0.386	0.322	0.354	0.380	0.314	0.347	0.396	0.314	0.355
Salicylic acid (1000 ppm)	0.396	0.336	0.366	0.364	0.309	0.336	0.367	0.312	0.340
Ethrel (800 ppm)	0.405	0.357	0.381	0.394	0.348	0.371	0.397	0.316	0.356
Maleic hydrazide (2000 ppm)	0.320	0.344	0.332	0.304	0.305	0.305	0.324	0.314	0.319
Mean	0.382	0.356		0.369	0.372		0.381	0.376	
CD (P=0.05)	0.008	0.010		0.005	0.006		0.003	0.004	
For pooled analysis CD (P=0.05)	S	0.005		S	NS		S	0.003	
	T	0.006		T	0.004		T	0.002	
	S x T	0.009		S x T	0.005		S x T	0.003	

Table 38. Effect of sterility inducing chemicals and growth regulators on ascorbic acid oxidase activity (Enzyme units g^{-1} min^{-1}) in leaves of TS 29 at panicle development and flowering stages

Treatments	Panicle development						Flowering		
	Five days after first treatment (Stage III)			Five days after second treatment (Stage V)					
	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean
Control	2.84	2.97	2.90	3.12	3.15	3.13	2.96	2.66	2.81
Salicylic acid (800 ppm)	3.92	4.86	4.39	4.65	6.12	5.39	5.44	6.55	5.99
Salicylic acid (1000 ppm)	4.02	4.87	4.44	4.95	6.12	5.54	5.38	6.44	5.91
Ethrel (800 ppm)	3.61	4.71	4.16	4.33	5.97	5.15	4.93	6.32	5.62
Maleic hydrazide (2000 ppm)	3.84	4.65	4.25	4.27	5.63	4.95	4.64	6.00	5.38
Mean	3.64	4.41		4.26	5.40		4.67	5.60	
CD (P=0.05)	0.026	0.020		0.032	0.029		0.045	0.036	
For pooled analysis CD (P=0.05)	S	0.013		S	0.032		S	0.026	
	T	0.015		T	0.029		T	0.027	
	S x T	0.022		S x T	0.022		S x T	0.038	

Table 39. Effect of sterility inducing chemicals and growth regulators on catalase activity (Enzyme units x 10^4 g^{-1} min^{-1}) in leaves of TS 29

at panicle development and flowering stages

Treatments	Panicle development						Flowering		
	Five days after first treatment (Stage III)			Five days after second treatment (Stage V)					
	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean
Control	2.94	2.84	2.89	3.64	3.61	3.62	3.54	3.55	3.55
Salicylic acid (800 ppm)	2.43	2.32	2.38	2.12	2.11	2.07	2.26	2.11	2.19
Salicylic acid (1000 ppm)	2.43	2.40	2.42	2.14	2.60	2.13	2.36	2.10	2.23
Ethrel (800 ppm)	2.54	2.59	2.57	2.33	2.64	2.47	2.43	2.66	2.54
Maleic hydrazide (2000 ppm)	2.54	2.58	2.56	2.43	2.60	2.53	2.44	2.96	2.70
Mean	2.58	2.55		2.53	2.60		2.61	2.68	
CD (P=0.05)	0.017	0.006		0.024	0.076		0.028	0.034	
For pooled analysis CD (P=0.05)	S	0.006		S	0.030		S	0.017	
	T	0.008		T	0.038		T	0.021	
	S x T	0.012		S x T	0.053		S x T	0.029	

Table 40. Effect of sterility inducing chemicals and growth regulators on peroxidase activity (Enzyme units l^{-1} hr^{-1}) in leaves of TS 29 at panicle development and flowering stages

Treatments	Panicle development						Flowering		
	Five days after first treatment (Stage III)			Five days after second treatment (Stage V)					
	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean
Control	163.42	161.22	162.32	176.49	174.54	175.51	184.41	182.59	183.50
Salicylic acid (800 ppm)	155.50	153.40	154.45	141.48	136.70	139.09	143.32	142.35	142.83
Salicylic acid (1000 ppm)	157.65	156.63	157.14	140.69	140.05	140.37	145.42	142.28	143.85
Ethrel (800 ppm)	154.58	158.74	156.66	153.99	151.66	152.82	154.45	162.48	158.46
Maleic hydrazide (2000 ppm)	160.42	156.44	158.43	151.69	152.97	152.33	155.37	160.25	157.81
Mean	158.31	157.29		152.87	151.18		156.59	157.99	
CD (P=0.05)	0.454	0.314		0.878	1.425		0.118	1.370	
For pooled analysis CD (P=0.05)	S	0.208		S	0.723		S	0.793	
	T	0.261		T	0.793		T	0.651	
	S x T	0.369		S x T	1.121		S x T	0.921	

Table 41. Effect of sterility inducing chemicals and growth regulators on superoxide dismutase activity (Enzyme units mg^{-1} protein hr^{-1}) in leaves of TS 29 at panicle development and flowering stages

Treatments	Panicle development						Flowering		
	Five days after first treatment (Stage III)			Five days after second treatment (Stage V)					
	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean
Control	8.44	8.25	8.34	8.49	8.39	8.44	8.50	8.45	8.43
Salicylic acid (800 ppm)	6.34	6.34	6.34	5.01	5.25	5.13	4.97	5.27	5.12
Salicylic acid (1000 ppm)	6.04	6.39	6.22	4.98	5.33	5.15	4.53	5.34	4.94
Ethrel (800 ppm)	7.57	7.33	7.45	5.33	5.96	5.64	5.97	5.99	5.98
Maleic hydrazide (2000 ppm)	7.63	7.41	7.52	6.73	5.97	6.35	6.01	6.34	6.17
Mean	7.20	7.14		6.11	6.18		6.00	6.28	
CD (P=0.05)	0.035	0.028		0.016	0.039		0.034	0.024	
For pooled analysis CD (P=0.05)	S	0.013		S	0.016		S	0.007	
	T	0.021		T	0.040		T	0.020	
	S x T	0.029		S x T	0.013		S x T	0.028	

Table 42. Effect of sterility inducing chemicals and growth regulators on the nutrient status in leaves of TS 29 during booting stage

Treatments	Potassium content (%)			Calcium content (µg g^{-1})			Copper content (µg g^{-1})			Boron content (µg g^{-1})		
	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean
Control	1.04	1.03	1.04	208.39	203.18	205.78	118.61	117.42	118.02	38.49	38.82	38.65
Salicylic acid (800 ppm)	1.03	1.03	1.03	161.23	141.05	151.14	81.58	86.21	83.39	22.64	20.42	21.53
Salicylic acid (1000 ppm)	1.04	1.03	1.03	163.08	143.45	153.26	81.50	82.66	82.08	23.22	21.51	22.36
Ethrel (800 ppm)	1.04	1.03	1.03	171.90	161.45	166.68	90.80	90.68	90.80	22.45	22.42	22.43
Maleic hydrazide (2000 ppm)	1.04	1.03	1.04	174.10	166.10	170.10	93.33	94.49	93.91	22.26	24.57	23.41
Mean	1.04	1.03		175.74	163.05		93.19	94.29		25.82	25.55	
CD (P=0.05)	NS	NS		1.103	2.942		2.002	0.961		0.132	0.215	
For Pooled Analysis CD (P=0.05)	S	NS		S	1.479		S	1.057		S	0.171	
	T	NS		T	1.488		T	1.052		T	0.009	
	S x T	NS		S x T	2.104		S x T	1.488		S x T	0.169	

Table 43. Effect of sterility inducing chemicals and growth regulators on nutrient status in panicles of TS 29 during booting stage

Treatments	Potassium content (%)			Calcium content (µg g^{-1})			Copper content (µg g^{-1})			Boron content (µg g^{-1})		
	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean	Season I	Season II	Pooled mean
Control	0.79	0.81	0.80	180.50	177.65	179.08	90.85	90.75	90.79	33.00	31.70	32.35
Salicylic acid (800 ppm)	0.46	0.53	0.50	125.85	111.93	118.89	65.87	60.76	63.31	15.72	14.22	14.97
Salicylic acid (1000 ppm)	0.51	0.59	0.55	123.53	108.35	115.94	66.52	66.46	66.49	14.52	14.92	14.72
Ethrel (800 ppm)	0.60	0.70	0.65	132.58	122.60	127.59	69.13	68.92	69.02	16.55	16.31	16.43
Maleic hydrazide (2000 ppm)	0.67	0.72	0.70	132.80	123.45	128.13	72.36	70.22	71.29	14.45	17.31	15.88
Mean	0.61	0.67		139.05	128.79		72.95	71.42		17.95	18.89	
CD (P=0.05)	0.011	0.012		2.561	2.313		0.735	0.668		1.383	0.510	
For Pooled Analysis CD (P=0.05)	S	0.006		S	1.271		S	0.510		S	0.764	
	T	0.008		T	1.634		T	0.471		T	0.698	
	S x T	0.011		S x T	2.311		S x T	0.666		S x T	0.987	