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Biochemical Basis of Thermosensitive Genic Male Sterility (TGMS) in Rice (*Oryza sativa* L.)

A. Senthil, R.C. Babu[1], M. Thangarajand[2] and N.M. Boopathi[1]

Department of Crop Physiology
Tamil Nadu Agricultural University
Coimbatore- 641 003, India.

ABSTRACT. *Biochemical basis of Thermosensitive Genic Male Sterility (TGMS) in rice was studied with three TGMS lines viz., TGMS 6, TGMS 16 and TGMS 29 along with a normal variety ADT 39. The plants were transferred to the phytotran at stage III of panicle development (25 days before heading). Different temperature treatments were given in the phytotran to simulate the diurnal variation in temperature of summer season for the induction of male sterility. The plants were kept inside the phytotran upto stage VII of panicle development. Leaf samples were collected between 10 to 25 days before flowering and young panicles were collected at 10 days before heading. Electrophoretic study of protein profile in leaves and anthers showed a quantitative difference between fertility and sterility. There existed a reduction in the larger subunit of Rubisco (54 kDa) and expression of smaller molecular weight protein (24 kDa) under sterility. Isozyme banding pattern of peroxidase (POX) and superoxide dismutase (SOD) revealed the presence of distinct isoforms with polymorphic nature under fertile and sterile conditions. These isoforms showed distinct differences between TGMS lines and normal variety. The presence of specific isoforms only in TGMS lines showed the potential of using the isozyme pattern as biochemical markers for screening TGMS trait in rice.*

INTRODUCTION

The hybrid technology in a strictly self-pollinated crop such as rice with an yield advantage of 2-3 t ha^{-1} over the best conventional varieties is a major landmark in the history of rice breeding. Inspite of their potential and promise, the conventional three-line hybrids are not without limitations. Of various weaknesses, the narrow genetic base for cytoplasmic male sterility (CMS), long cumbersome process of developing parental lines with desired agronomic features, need of maintainers for multiplication of A lines, utmost care to maintain the genetic purity of all the three lines and above all, the higher seed cost are important. These limitations have necessitated for alternative approaches to exploit hybrid vigour.

One of the possible alternatives is the two line breeding system, which comprises environmental sensitive genic male sterility (EGMS) (Ali, 1995). EGMS comprises photosensitive genic male sterility (PGMS) which is based on the variation in day length and thermosensitive genic male sterility (TGMS) which is caused by high/low temperature. TGMS system is more suitable wherein the genotypes becomemale sterile at particular temperature and become fertile at some other

1 Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore- 641 003, India

2 Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore- 641 003, India.

temperature (Borkakati and Virmani, 1996). Several TGMS lines have been identified and the temperature ranges inducing sterility / fertility are being standardized (Ilyas Ahmed, 1996). Though considerable information is available on the heterosis and combining ability of two line hybrids using TGMS, the change of fertility to sterility and vice-versa in TGMS lines should be quite clear and distinct. There is a need for understanding the molecular and biochemical basis of male sterility caused by high temperature. Studying the molecular and biochemical mechanisms of fertility/sterility transformations of TGMS lines would open new frontiers for manipulating deviation in fertility status under unexpected weather conditions.

The present study was carried out primarily to understand the biochemical and molecular basis of TGMS in rice by SDS – PAGE analysis of protein profiles under fertile and sterile conditions. Besides these, isozyme analyses of certain enzymes like peroxidase and superoxide dismutase were also done to understand their role in the regulation of fertility/sterility in TGMS lines.

MATERIALS AND METHODS

The work was carried out in Tamil Nadu Agricultural University, Coimbatore during 2000-2001. Three TGMS lines (TGMS 6, TGMS 16 and TGMS 29) along with a normal variety ADT 39 were chosen. Rice plants were grown in the glass house in plastic pots. The plants were transferred to the growth chamber (Phytotran), when they were in stage III of panicle development (25 days before heading). The temperature treatments were given in the growth chamber to simulate the diurnal variation of summer season for induction of male sterility.

Sowing date was adjusted so that the lines came to panicle initiation stage during 1st week of December. The mean maximum and minimum temperature for December and January were 29.4 and 17.9°C respectively. Five pots each with three plants were maintained. Fertile set of TGMS lines and ADT 36 were kept in the glass house. The plants were kept inside the growth chamber upto stage VII of panicle development. Leaf samples were collected between 10 to 25 days before flowering and young panicles were collected at 10 days before heading.

SDS-PAGE analysis was done to identify the qualitative and quantitative differences in the expression of proteins under sterile and fertile condition as per the method of Laemmli (1970). Isozyme analysis of peroxidase (POX) and superoxide dismutase (SOD) was done as per the procedures proposed by Sadasivam and Manickam (1996). The Isozyme bands of each enzyme were designated by numbers corresponding to their relative mobility (Rm) related to the dye front.

RESULTS AND DISCUSSION

Protein profile in TGMS lines under sterile and fertile conditions

The results of SDS-PAGE protein profile of TGMS lines showed that there existed quantitative difference in the expression of proteins under fertile and sterile conditions (Figure 1). Under fertile condition the leaves of all the three TGMS lines *viz.*, TGMS 6, TGMS 16, and TGMS 29 showed an enhanced expression of 64, 54 and 24 kDa proteins, while in anthers 48, 38 and 22 kDa proteins were expressed in higher

intensity (Figure 2). Under sterile condition, the above said proteins were expressed in lower intensity in both the samples, but a protein of 14 kDa was expressed under sterile condition only in anthers.

The lower intensity in the expression pattern of 54 kDa protein profiles in leaves of TGMS lines under sterile condition was due to impaired synthesis of larger subunit complex of Rubisco under stress (Hidema *et al.*, 1992). This was also confirmed by the results of Miller (1988) who reported the quantitative change in Rubisco under high temperature, since it was coded by chloroplast genome. High temperature stress caused membrane damage, protein denaturation and mutation of DNA (Bowler *et al.*, 1992).

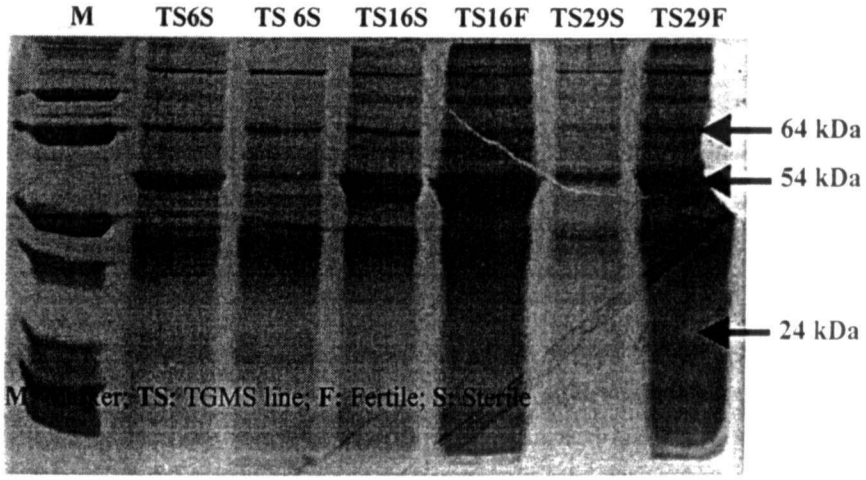

Fig. 1. Leaf protein profile of TGMS lines under fertile and sterile conditions.

Note : M: Marker; TS: TGMS line; F: Fertile; S: Sterile

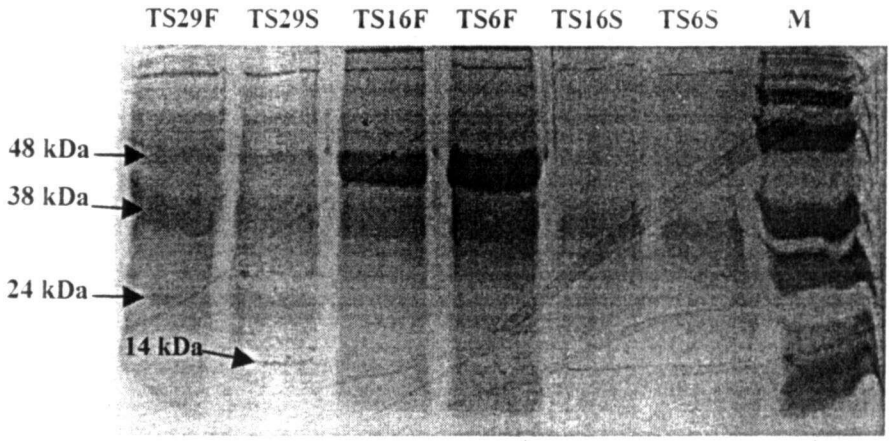

Fig. 2. Anther protein profile of TGMS lines under fertile and sterile conditions.

Note : M: Marker; TS: TGMS line; F: Fertile; S: Sterile

This might be also the reason for lower expression of other proteins in sterile condition. Enhanced expression of proteins of 64 and 24 kDa was seen in leaves of fertile plants of TGMS lines. This was in accordance with the findings of Roystephen and Thangaraj (2000). Compared to sterile TGMS lines, the higher intensity of 48, 38 and 24 kDa protein bands in fertile TGMS lines suggested their role in fertility expression in TGMS lines and the presence of smaller molecular weight protein (14 kDa) might be associated with the expression of male sterility genes in TGMS lines. The characterization of protein, which expressed under sterile condition, will be useful to understand the expression of male sterility genes in TGMS lines. Huang *et al.* (1994) reported the differences in protein components of anthers of PGMS lines under fertile and sterile conditions. They have observed two specific bands at 43 and 40 kDa in fertile anthers. However, in the present study, TGMS lines expressed 2 high intensity protein bands at 48 and 38 kDa in fertile anthers. These findings confirmed the reports of Limei Ru *et al.* (1999) that the proteins at the isoelectric focus point were associated with fertility expression during panicle development. This result suggested the possible role of these proteins in fertility and sterility expression in TGMS lines.

Isozyme analysis of certain enzymes

Isozyme patterns of TGMS lines were analysed under fertile and sterile conditions in comparison with a normal variety (ADT 39) to detect the polymorphism. Isozymes have a definite role on regulating plant growth and development (Li *et al.* 1994). Freeling (1983) observed that isozyme patterns were the better biochemical means to analyse the interactions with higher accuracy. Studying the changes in the expression of certain enzymes such as peroxidase (POX) and superoxide dismutase (SOD) also play a crucial role in the regulation of fertility/sterility in TGMS lines, which needs further confirmation.

Peroxidase (POX)

Isozyme pattern of POX revealed the presence of specific isoforms under fertile and sterile conditions in TGMS lines (Figure 3). Liu *et al.* (1993) reported that the isozyme band number and intensity were different under fertile and sterile conditions. Li *et al.* (1993) also observed the difference in POX isozyme banding pattern of fertile and sterile PGMS lines with two extra bands under fertile condition. In the present experiment also, there were four extra isoforms in TGMS lines under fertile condition. The POX 2 was associated with fertility of TGMS lines which was also present in the normal variety ADT 39, but it was absent in sterile TGMS lines. Wu and Xiao (1993) found variation in POX isozyme banding pattern and its activity in TGMS lines. A specific band POX 1 was present only in TGMS lines with uniform high activity. Elsy (1997) reported a similar isozyme banding pattern of POX under both high and low temperatures. In this experiment POX 1, 3 and 4 were present in all the TGMS lines both under fertile and sterile conditions.

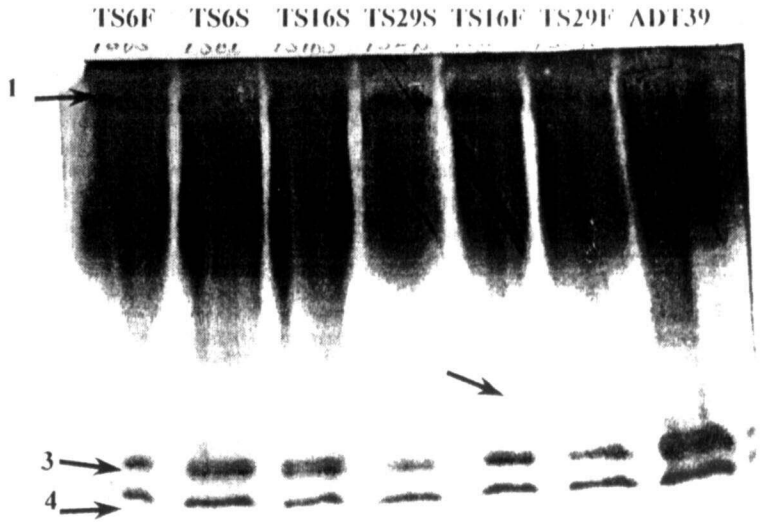

Fig. 3. **Isozyme pattern of peroxidase (POX) in leaves of TGMS lines under fertile and sterile conditions.**
Note : TS: TGMS line; F: Fertile; S: Sterile

Superoxide dismutase (SOD)

The isoenzymatic pattern of SOD showed the presence of more bands (4 bands) under fertile condition than under sterile condition (3 bands) in TGMS lines (Fig.4). Fertile TGMS lines yielded one extra band regardless of genotypes and the intensity of the band was more compared to the sterile lines. The results of the present study was in support of the findings of Zhang *et al.*, (1994) who observed higher SOD activity under fertile condition compared to sterility in TGMS lines.

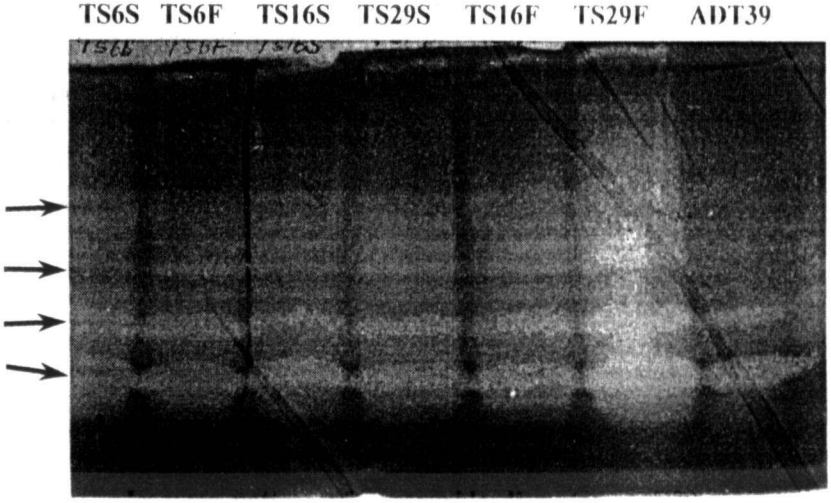

Fig. 4. **Isozyme pattern of superoxide dismutase (SOD) in leaves of TGMS lines under fertile and sterile conditions.**
Note : TS: TGMS line; F: Fertile; S: Sterile

For TGMS lines high temperature was an adversity, which produced more reactive oxygen species causing membrane damage, change in structure and function of DNA and RNA and denaturation of proteins. This might be the cause for the absence of certain isozymes and the lesser intensity of isoforms present under sterile condition.

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