# CLONING AND CHARACTERIZATION OF A TAPETUM SPECIFIC GENE FROM TOMATO

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TA29 is an anther specific gene in tobacco which has been widely used for engineering male sterility in tobacco. A homologue of TA29 gene was isolated from tomato by PCR using tomato genomic DNA as template. Primers specific for TA29 gene were designed on the basis of available sequence from tobacco. Use of these primers in PCR, amplified a product of expected size that was cloned in TA cloning vector and was completely sequenced. Sequence analysis showed that gene from tomato has high sequence homology to tobacco TA29 gene. Homology search also showed similarities to cDNA clone from some other solanaceous plants including capsicum, petunia and potato. The conserved nature of this gene in tomato and tobacco suggests that the gene can be reliably targeted both in tobacco and tomato for engineering male sterility.
**Keywords:** Tapetum, anther specific genes, male sterility, tobacco, tomato

## INTRODUCTION

During sporogenesis in flowering plants different cell layers of anthers like stomium, connective and tapetum play important role to make functional and mature pollen. Among these layers tapetum plays an important role in pollen wall development and release of pollen grains from tetrad condition after meiosis (Pacini *et al*., 1985). Male fertility specific genes like TA29 are expressed specifically in the tapetum layer of anthers (Koltunow *et al*., 1990; Evrard *et al*., 1991; McCormick, 1991; Scott *et al*., 1991). Many tapetum specific genes have been cloned and their effect on male sterility has been studied. The isolation of anther development gene provides useful entry points into pollen differentiation and controlling points for microsopore development. In different studies anther-cell specific promoters have been identified, cloned and fused to cytotoxic structural genes, such as barnase genes to selectively destroy the anther tissue (Mariani *et al* 1990). This work laid the foundation for molecular and genetic approaches that helped in identification of genes which encode different cell-specific functions and are involved in developmental pathways. The silencing of such genes is ultimately helpful in hybrid seed production, where male sterility is prerequisite to start hybrid development program. These anther specific genes are conserved mainly across different species. TA29 is an anther specific gene in tobacco which is not transcribed detectably in other plant tissues (Koltunow *et al*., 1990 and Mariani *et al*., 1990, 1992). So far no concerted effort has been made to clone and characterize tapetum specific gene from tomato. We are developing a system to isolate, clone, characterize and perform functional studies on male specific genes from tomato. Here we have cloned a homologue of TA29 gene from tomato and showed that the gene is highly similar to TA29 gene in tobacco.

## MATERIALS AND METHODS

The sequence of TA29 gene from *Nicotiana tobaccum* was downloaded from NCBI (Accession number X52283.1). The primers were designed on the basis of GC rich contents and also keeping in view the restriction sites for cloning.

The sequence of the primers along with restriction sites for forward primer (TA29F) was 5'CTCGAGTGTGTTGCATTGGAGAAACA3' and for the reverse primers (TA29R) the sequence was 5'CCATGGTTGCACTGTCCCTGGCTTTG3'.

Total nucleic acids were extracted from tomato leaf samples by the CTAB method (Doyle and Doyle, 1989). Total isolated DNA from tomato was quantified by DyNA Quant200 flourometer. Fermentas PCR kit was used for amplification of gene. The profile used in the PCR reaction was 94, 50 and 72$^0$C respectively for denaturation, annealing and primer extension, each for 1 minute in the eppendorf thermal cycler run for 40 cycles. The amplified PCR fragment was resolved on 1% agarose gel and then visualized under UV light to confirm its size. PCR products were cloned directly into the plasmid vector pTZ57R/T as recommended by the manufacturer (Fermentas). For each, 20µl reaction, the reaction mixture include, 6µl of PCR product, 1µl pTZ57R/T, 1µl DNA Ligase, 2µl 10× Ligase buffer and finally 20µl volume was achieved by adding nuclease free water. The PCR product thus cloned in pTZ57R/T was directly used as a template in sequencing reaction. The complete nucleotide sequences of TA29 clone was determined by dideoxynucleotide chain-termination sequencing using the PCR-based BIG DYE

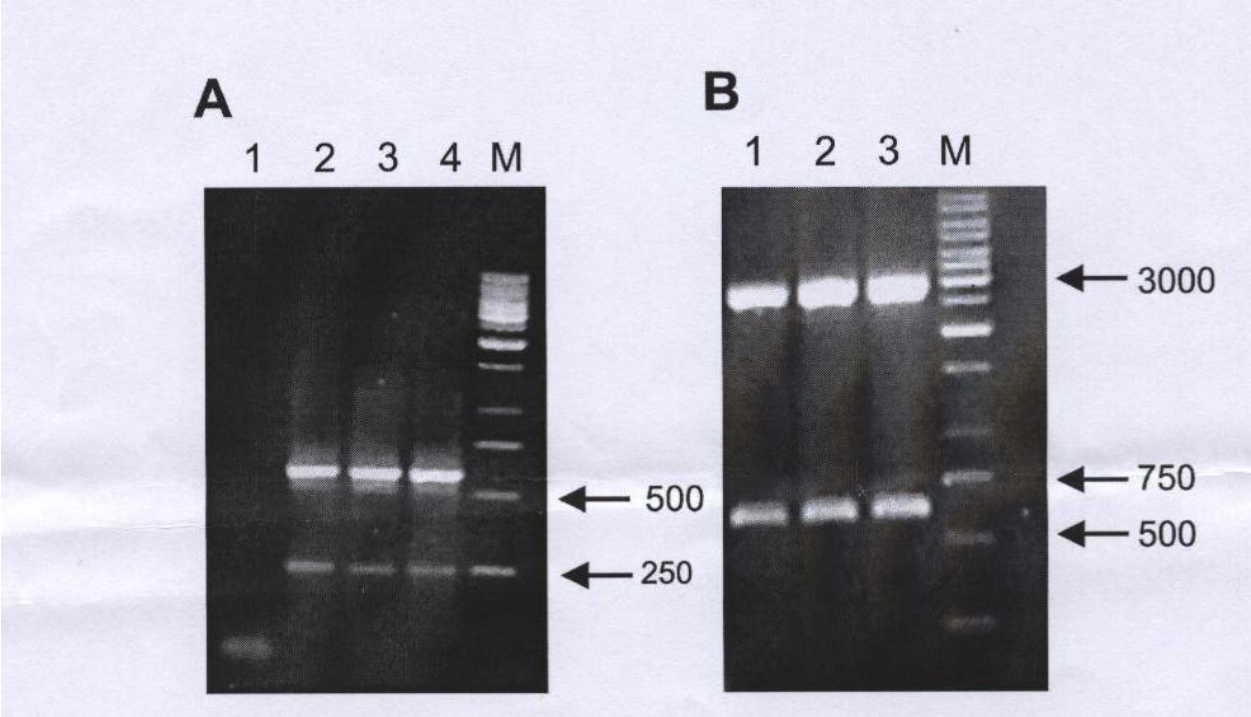


**Figure 1.** Stages in the production of construct for TA29 gene in PTZ57R/T. **Panel A** shows the approximately 550 bp products of amplifications with primers TA29f and TA29r (Lane 2). A molecular weight size marker is run in lane M. The sizes of selected marker bands (bp) are indicated. **Panel B** shows the cloning of TA29 in PTZ57R/T vector. The clones in lane 1, 2 and 3 shows the TA29 clone in PTZ57R/T restricted with *Eco*RI and *Pst*I.

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TA29Tobacco     FGVPVYSPGCGYVCPADIPTGGMTESKITGISQSARLYRCKPGPNMCDSKDCNELLLHFV
TA29Tomato      ----------------------MTESKITGISQSARLYRCKPGPNMCDSKDCNELLLHFV
                                       ************************************

TA29Tobacco     FPMQDKHDNKQEHLRYGGRRGIGLTVGGVGGFGIGFGAWGGGGGGGGGGSDAPGCSNDGC
TA29Tomato      FPMQDKHDNKQEHLRYGGRRGIGLTVGGVGGFGIGFGAWGGGGGGGGGGSDAPGCSNDGC
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TA29Tobacco     DPGFGCPPGCGYACPANNPSGGITEFHISGLSRFDGPYRCRPDMCESEDCNELLLHFVSP
TA29Tomato      DPGFGCPPGCGYACPANNPSGGITEFHISGLSRFDGPYRCRPDMCESEDCNELLLHFVSP
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TA29Tobacco     MQHKHX
TA29Tomato      MQHIX-
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**Figure 2.** Protein sequence comparison of tobacco and tomato TA29 genes. The stars show the sequence homology between the amino acid sequences of both the genes. Note that there are more number of glycine amino acids (G).

kit (Perkin-Elmer Cetus). Reaction products were resolved on ABI 3700 automated sequencer. Sequence information was stored, assembled and analyzed using version 7 of the program library of the Genetics Computer Group (Devereaux *et al*., 1984). Sequence alignments were produced by using DNA STAR software version 5 in CLUSTAL W program.
Homology search for sequence of TA29 clones was performed using basic local alignment sequence tool (BLAST) at web http://www.ncbi.nlm.nih.gov/blast.

## RESULTS

In the PCR reaction there was amplification of nearly 550 bases for the TA29 gene in tomato and tobacco. In both tobacco and tomato there was a bright band which showed similar size on ethidium stained agarose gel under UV light (Figure 1A). PCR product from tomato was cloned and confirmed by restriction with *Eco*RI and *Pst*I that showed only two bands, one each for vector and inserted fragment (Figure 1B). The clone from tomato was completely sequenced and was compared with DNA sequence in the data base. The sequence showed the exact length of 515 base pairs (bp) with one open reading frame of 489 base pairs. The sequence comparison with tobacco TA29 product showed 98.2% similarity by CLUSTAL W program. By BLAST analysis the sequence showed maximum homology with tobacco TA29 clone.

## DISCUSSION

Our results showed that TA29 gene is highly conserved in tobacco and tomato. The NCBI BLAST program was used to further explore other sequences with homology to TA29. Established sequence tags (EST's) available in the database for different plant species were also compared. In BLAST analysis about 200 bases for 3' end showed maximum homology with different floral cDNA isolates from plant species belonging to family *solanaceae*. This sequence was 86% similar to C*apsicum annum* cDNA clone (Accession number BM068405.1), 87% similar with floral cDNA clone of potato (Accession number CV50979.1) and 85% similar with petunia cDNA clone (CV295951.1). These results suggest that TA29 is conserved among several plants belonging to the same family.
The translated TA29 protein from tomato is glycine rich in nature. The presence of glycine in male specific genes is well documented in many plant species including rice (Jeon *et al*., 1999). TA29 and many other genes like TA13 are also very similar to each other at nucleotide level (Goldberg, 1988). Both of these genes are expressed in tapetal cell layer of tobacco flower and have about similar copies in the genome (Mariani *et al*., 1990). Previous observations by Seurinck *et al*, (1990) and our TA29 sequence analysis showed that this gene contains glycine rich protein without any intron.
TA29 gene has been targeted for engineering male sterility in tobacco (Mariani *et al*., 1990). Highly conserved nature of TA29 in tobacco and tomato suggest that the gene can be reliably targeted for engineering of male sterility in both tomato and tobacco. We have recently shown that targeting of TA29 through RNA interference (RNAi) results in male sterility (Mansoor *et al*., 2006). Our clone in pTZ57R/T with restriction sites can be used for further cloning in any gene silencing vector and can be used for induction of male sterility in crop plants.

### Accession Number

This sequence was named as TA29SN1 and accession number for this clone is AM261325 in EMBL (European molecular biology laboratory) data base.

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