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Research article

# Understanding the role of DNA polymerase λ gene in different growth and developmental stages of *Oryza sativa* L. indica rice cultivars

Sayantani Sihi [a], Soumitra Maiti [a], Sankar Bakshi [b], Arup Nayak [a], Shubho Chaudhuri [c], Dibyendu Narayan Sengupta [a, *]

[a] Division of Plant Biology, Bose Institute, 93/1 A.P.C. Road, Kolkata 700009, India
[b] Vidyasagar College for Women, 39 Sankar Ghosh Lane Kolkata 700006, India
[c] Division of Plant Biology, Bose Institute, P-1/12, C.I.T. Scheme VIIM, Kankurgachi, Kolkata 700054, West Bengal, India

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ABSTRACT

DNA polymerase λ (Pol λ) is the only member of DNA polymerase family X present in plants. The enzyme is ddNTP sensitive as it contains the conserved C-terminal Pol β domain. The 1.1 kb partial coding sequence isolated spanned the whole 3′ regions of the gene containing functionally important domains of the Pol λ gene. Comparative *in silico* studies from both indica and japonica cultivars involving homology modelling showed that the model for the partial Pol λ gene was stable and acceptable. The alignment of both the protein models showed a RMS value of 0.783.

Apart from this, expression of Pol λ and its relative activity is studied during different development stages of three different indica rice cultivars (IR29, Nonabokra and N22). Enhanced accumulation and higher activity of Pol λ during the early seedling stage was detected. Higher expression and activity were observed in the anthers, which was probably necessary for DNA repair during microspore formation. However, during the maturation stage of seed development and plant growth, expression and the activity of Pol λ decreased due to slow metabolic activity and a reduced rate of cell division respectively. Furthermore, the expression and activity of Pol λ were found to be higher in IR29 in comparison to Nonabokra and N22. IR29 is a rice cultivar susceptible to environmental stresses and hence it encounters higher DNA damages. The enhanced presence and activity of the Pol λ enzyme in IR29 with respect to the other two cultivars, which are more tolerant to the environmental stresses during various developmental stages, is therefore explainable.



## 1. Introduction

Rice (*Oryza sativa* L.) is a major agronomic crop, and approximately one-fifth of the world's population depends on rice cultivation for their livelihoods (https://www.irri.org). India is one of the world's biggest producers of rice, accounting for 20% of total global rice production. Moreover, India has the largest area under rice cultivation. It is cultivated throughout the year and thus, as a crop, rice is exposed to a diverse set of environmental stresses, both biotic and abiotic, which affect the crop yield. Most of the abiotic environmental stresses are known to cause DNA damage and hamper the genome integrity of the plant.

In living organisms, DNA polymerases are required for three critical cellular processes: DNA replication, repair and recombination (Furukawa et al., 2015). Based on their structures and functions, the DNA polymerases have been grouped into seven families – A, B, C, D, X, Y and reverse transcriptase. Six members of DNA polymerase family X – Pol β, Pol λ, Pol μ, Terminal deoxynucleotidyltransferase (Tdt), Pol $\sigma_1$ and Pol $\sigma_2$ have been identified from the eukaryotic system (Ramadan et al., 2004). Members of DNA polymerase family X are conserved in most organisms from bacteria to humans (Uchiyama et al., 2009; Oliveros et al., 1997). But despite their conserved nature, Pol λ is the only member of DNA polymerase family X present in the plant systems. *Arabidopsis* and *Oryza sativa* have been used as model plants to study the function of plant Pol λ (García-Díaz et al., 2000; Uchiyama et al., 2004; Amoroso et al., 2011; Roy et al., 2011). At the amino acid level, Pol λ shows a 30% homology with Pol β. The C-terminal domain of Pol λ

*Abbreviations:* cds, Coding sequence; ddNTP, Dideoxynucleotidetriphosphate; PMSF, Phenylmethanesulfonyl fluoride; PVDF, Polyvinylidenedifluoride.

* Corresponding author.
*E-mail address:* dnsenguptabi@gmail.com (D.N. Sengupta).

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is similar to Pol β, whereas the N-terminal part of Pol λ has BRCT (breast cancer type 1 susceptibility protein C terminus) domain, known for interactions with other BRCT-domain-containing proteins (Roy et al., 2009). Pol λ is a single polypeptide, ddNTP sensitive, aphidicolin insensitive DNA repair protein. It is involved in base excision repair (García-Díaz et al., 2001), non-homologous end joining (Lee et al., 2004) and translesion synthesis (TLS) for repairing oxidative DNA damage (Maga et al., 2007). Although the biochemical properties of Pol λ are widely studied in plants, its *in vivo* functions are hardly studied and understood. Earlier studies demonstrated the role of OsPolλ in BER (Sarkar et al., 2004) and increased transcript abundance in culture cells when exposed to UV-B irradiation and MMS treatment, suggesting the role of Pol λ in repairing methylated bases (Uchiyama et al., 2004).

Germination of seeds involves a series of events that start with the uptake of water by quiescent dry seeds and terminates with the elongation of the embryonic axis (Bewley and Black, 1994). The metabolic machinery is present in dormant dry seeds in an inactive form. Upon imbibition, the dry seeds rapidly resume their metabolic activity resulting in a division of embryonic cells and seed germination (Bewley, 1997). Fragmentation of nuclear DNA occurs during the maturation, drying and long dehydration period in dry seeds (Cheah and Osborne, 1978). In plants, for survival, proper cell division is required which can occur only after the repair of damaged DNA.

The present work deals with the study of gene expression and enzyme activity of the Pol λ during different growth and developmental stages in three different indica rice cultivars. Comparative study of Pol λ in these three indica rice cultivars - IR29, Nonabokra and N22 – will be helpful in giving us an insight into the role and function of Pol λ during different vegetative and reproductive developmental stages. This study will further elucidate the critical role and involvement of DNA polymerase λ in controlling various cellular and molecular activities as well as the physiological effect.

## 2. Materials and methods

### 2.1. Plant materials and growth conditions

Seeds of *Oryza sativa cv.* IR29 were collected from CRRI, West Bengal; *cv.* Nonabokra from CSSRI, West Bengaland N22 from NIPGR, New Delhi. Seeds were multiplied at the Madhyamgram Experimental Farm (MEF) of the Bose Institute, West Bengal, India. Seeds were surface sterilized with 0.1% $HgCl_2$, and after thorough washing, the seeds were imbibed overnight in sterile water and then spread over moist gauge cloth in plastic trays. Seeds were allowed to germinate in autoclaved deionized water at 37 °C in darkness for 3 days. Plants were grown for 8 weeks in greenhouse conditions (photoperiod: 16/8 h, temperature: 28 ± 3 °C) and were transferred to an experimental field (MEF) to procure large amounts of the crop. Plant tissues were collected at different stages of development and from various organs. All samples were collected from three consecutive years and experiments were performed with three biological and technical replicas.

### 2.2. Amplification and cloning of partial coding sequence of Pol λ gene

Total RNA was isolated from 8-day-old seedlings of IR29, Nonabokra and N22 cultivars by the Phenol–SDS method (Longhurst et al., 1994). After DNase I (Roche) treatment, cDNA was prepared using Sensiscript RT Kit (Qiagen, USA) and was used as a template for PCR amplification using Taq polymerase (Genet Bio). Actin was used as constitutive gene and was amplified using forward primer ActinF-5′CCTCATGAAGATCCTGACGG3′ and reverse primer ActinR-5′GGAATGTGCTGAGAGATGCC3′. For full length Pol λ cDNA amplification, different oligonucleotides were designed according to the *Oryza sativa* japonica sequence (Uchiyama et al., 2004) but without much success except for one set of partial primer, i.e. forward primer PolEx3F-5′GCCAAAGTGCCTCTGGAGAT3′ and reverse primer PolEx14R-5′CAGTCTCGAGCTAGAGATTACGTTCGTGAGGTTC3′. PCR was performed for 25 cycles of 45 s at 94 °C, 30 s at 60 °C (annealing temperature) and 90 s at 72 °C with final extension step for 20 min at 72 °C. The PCR product was purified using QIAgen gel extraction kit (QIAGEN, USA). The purified PCR product was ligated with TA vector (Thermo Scientific) and transformed into competent *E. coli* (strain DH5α; Clontech, USA) cells. Transformed cells were screened by colony PCR. The plasmids from the selected positive colonies were isolated. The recombinant clones were confirmed by restriction digestion, and sequencing was carried out using M13 forward and M13 reverse primers according to the standard manufacturer protocol of Applied Biosystem, USA. The obtained nucleotide sequence and its transformed amino acid sequence were analysed using different *in silico* tools to confirm it as part of DNA polymerase λ.

### 2.3. Multiple sequence alignment and amino acid sequence analysis of Pol λ gene

The sequences of the amplicons were used as query sequences for BLAST searches in NCBI. The sequences of Pol λ reported from *Oryza sativa* japonicar and the amplified partial coding sequence from the indica rice were subjected to multiple sequence alignments using Clustal Omega to ascertain the presence of Single Nucleotide Polymorphisms (SNPs) in the indica Pol λ gene. The partial coding sequence was then converted to an amino acid sequence using Expasy translate tools (http://www.expasy.org/). It was used as a query for conserved domain search (Marchler-Bauer et al., 2015; Marchler-Bauer et al., 2011; Marchler-Bauer et al., 2009; Marchler-Bauer and Bryant, 2004).

### 2.4. Homology modelling and structural analysis of Pol λ gene

The protein model of the partial indica Pol λ was generated using the SWISS-MODEL (Guex and Peitsch, 1997) package provided by the Swiss-PDB viewer program based on the crystal structure of DNA polymerase λ (PDB ID:3c5g.2.A) as the template. The model quality was assessed using PROCHECK (Liang et al., 1998). The stereochemical stability of the model was checked and a Ramachandran plot for the model was obtained.

### 2.5. Genomic DNA isolation and southern blot analysis

Genomic DNA was isolated from the plumules of IR29, Nonabokra and N22 germinated seedlings by the cetyltrimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980). DNA (10 μg) was digested with *Eco*RI at 37 °C and electrophoresed on 0.8% agarose gel (Sambrook and Russell, 2001). The gel was initially treated with HCl). After repeated washing with deionized water, the gel was soaked in denaturation solution (1.5 M NaCl, 0.5 M NaOH) followed depurination solution (0.2 N by brief rinsing with deionized water. The gel was subsequently immersed in a neutralization buffer (1 M Tris-Cl pH7.4, 1.5 M NaCl) until the pH of the buffer came below 7.5. The DNA was transferred to a Nytran membrane (GE, Amersham) by capillary transfer in 10X SSPE (Sigma Aldrich) solutions for overnight. The membrane was cross-linked by UV radiation in UV cross linker (Hoefer, UVC 500) for 2 min under 12,000μJ/sq. cm (Church and Gilbert, 1984). It was incubated in a prehybridization solution for 6 h at 42 °C and then hybridized with the radiolabelled probe (specific activity