Detection of DNA polymerase λ activity during seed germination and enhancement after salinity stress and dehydration in the plumules of indicarice (Oryza sativa L.)

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DNA polymerase λ (DNA pol λ) is the only reported X-family DNA polymerases in plants and has been shown to play a significant role in dry quiescent seeds, growth, development and nuclear DNA repair. cDNA for DNA pol λ has been reported in Arabidopsis and japonica rice cultivar and has been characterized from E. coli expressed protein, but very little is known about its activity at protein level in plants. The enzymatic activity of DNA pol λ was studied in dry, imbibed and during different germination stages of indica rice IR-8 (salt sensitive) by in-gel activity assay to determine its physiological role in important stages of growth and development. The upstream sequence was also analyzed using plantCARE database and was found to contain several cis-acting elements, including light responsive elements, dehydration responsive elements, Myb binding sites, etc. Hence, 4-day-old germinating seedlings of IR29, a salt-sensitive, but high yielding indica rice cultivar and Nonabokra, a salt-tolerant, but low yielding cultivar were treated with water (control) or 250 mM NaCl or 20% polyethylene glycol-6000 for 4 and 8 h. The protein was analyzed by in vitro DNA pol λ activity assay, in-gel activity assay and Western blot analysis. DNA pol λ was not detected in dry seeds, but enhanced after imbibition and detectable from low level to high level during subsequent germination steps. Both salinity and dehydration stress led to the enhancement of the activity and protein level of DNA pol λ, as compared to control tissues. This is the first evidence of the salinity or dehydration stress induced enhancement of DNA pol λ activity in the plumules of rice (Oryza sativa L.) cultivars.

Keywords: Oryza sativa indica rice, DNA pol λ, ddNTP sensitivity, Activity gel, Abiotic stress

The enzymes that synthesize DNA using a template DNA strand and the 3'-OH end of a nucleic acid (RNA/DNA) as a primer are known as DNA polymerases. Based on their structures and functions, they have been grouped into seven DNA polymerase families – A, B, C, D, X, Y and reverse transcriptase. The mitochondrial DNA polymerase γ belongs to the A family and eukaryotic DNA polymerases α, δ and ε all belong to the B family. Six pol X-family members – Pol β, Pol λ, Pol μ, Terminal deoxynucleotidyl transferase (Tdt), Pol σ₁ and Pol σ₂ have been identified from eukaryotic system. At amino acid level DNA pol λ shows 30% homology with DNA pol β. The C-terminal domain of DNA pol λ is like pol β, whereas the N-terminal part of DNA pol λ has BRCT (breast cancer type 1 susceptibility protein C terminus) domain, known for interactions with other BRCT-domain containing proteins.

DNA synthesis is required both during replication and repair. Once the DNA repairing has been done, both efficient and high fidelity DNA polymerase is required for replication of DNA during active growth. DNA polymerases like DNA pol β (38 kDa) and DNA pol λ (62 kDa) in mammals have been reported to be involved in repair of genomic DNA. Previous studies have established that in mammalian system, DNA pol λ is involved in three major repair pathways—long patch base excision repair, non-homologous end joining (NHEJ) and trans lesion synthesis (TLS).

In plant system, pol λ has been identified and cloned from Arabidopsis and rice (Oryza sativa L.).

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Abbreviations: BRCT, breast cancer type 1 susceptibility protein C terminus; dCMP, deoxycytidine monophosphate; ddNTP, dideoxynucleotide triphosphate; DNA pol λ, DNA polymerase λ; DRE, dehydration responsive elements; DRR, DNA recombination and repair; HSE, heat shock elements; LRE, light responsive elements; PCNA, proliferating cell nuclear antigen; PEG6000, polyethylene glycol 6000; PMSF, phenylmethylsulfonyl fluoride; TCA, trichloroacetic acid; Tdt, terminal deoxynucleotidyl transferase.

† For Supplementary data, see www.nopr.niscair.res.in
Interestingly, DNA Pol λ has terminal deoxynucleotidyl transferase and deoxyribose phosphate lyase activities, in addition to DNA polymerase activity. The DNA Pol λ protein is found to be involved in base excision repair and its association with rice proliferating cell nuclear antigen (PCNA) is revealed by the pull down assay. The processivity of DNA Pol λ is found to be enhanced by PCNA. DNA damaging agents like methyl methane sulphonate are reported to induce the expression of DNA Pol λ gene. Two transcripts (splicing variants) are found to be abundant in shoot apical meristem and in panicle of rice.

The expression of pol λ gene has been detected at low level throughout the plants, although high level of its activity is reported in meristematic tissue and anthers. Further, we have characterized DNA Pol λ (previously known as DNA Pol β, since the enzyme is sensitive to ddNTP, but not to aphidicolin) from rice and Vigna radiata L. Singh et al. have reported the presence of DNA recombination and repair (DRR) genes from Arabidopsis and rice. Several functionally important DRR gene duplications are present in Arabidopsis, which do not occur in rice.

Rice is a major agronomic crop in Asia. In India, it is cultivated throughout the year and requires stagnant water in the Indo-gangetic plains and also in the upland areas during summer with irrigated water. Thus, as a crop, rice is exposed to a wide variety of environmental factors. Indica rice cultivars like Pokkali and Nonabokra are well-known for salt tolerance, whereas cultivars like N22 and Sahbhagi Dhan (IR 15) are tolerant to drought. However, all these cultivars are low yielding in comparison to the high yielding rice cultivars like Taichung native 1, IR 29, M-1-48, IR 8, etc.

In the literature, very scanty information is available about DNA Pol λ at protein level in rice and its activity in response to abiotic stress like salinity or dehydration stress. Here, we have investigated the level of DNA Pol λ in seeds during imbibition, germination stages and in the plumules of IR8, a salt-sensitive rice cultivar by activity gel and Western blot analysis. In addition to the investigation of DNA Pol λ during germination stages, its expression is also detected in the plumules only after salinity stress or dehydration. Further, we have compared salt-sensitive and salt-tolerant indica rice cultivars after treatment with salinity stress or dehydration. The significance of expression of DNA Pol λ at protein level has also been studied.

### Materials and Methods

#### Chemicals

- Agarose, polyvinylpyrrolidone (PVP), N,N'-methylenebisacrylamide, glycine, ethidium bromide, bovine serum albumin, tween-20, β-mercaptoethanol and dichemlinoethyl (DEAE) sephacel were obtained from Sigma-Aldrich. Acrylamide, polyvinylidene fluoride (PVDF) membrane were from GE Amersham-Pharmacia (USA), QIAquick nucleotide removal kit was from Qiagen (USA). Mercuric chloride, bromophenol blue, Luria-Bertani (LB) broth and agar, ethylenediaminetetraacetic acid, ammonium sulfate, ethanol, isopropanol, glycerol were from E. Merck (India & Germany). Special quality sodium dodecyl sulphate (SDS) was from Roche (Germany). Sodium bicarbonate, potassium chloride, sucrose, tris, sodium chloride, polyethylene glycol-6000, nitro blue tetrazolium chloride (NBT), 5-bromo-4-chloro-3-indolyl phosphate (BCIP), magnesium chloride, potassium acetate, phenylmethylsulfonyl fluoride (PMSF), sodium hydroxide, carinol, glacial acetic acid, trichloroacetic acid (TCA), calcium chloride, ampicillin, isopropyl β-D-1-thiogalactopyranoside (IPTG), 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-Gal), sulfoisalysic acid, hydrochloric acid, chloroform, dithiothreitol (DTT), cetyl trimethylammonium bromide (CTAB) and phenol were from SRL (India). Whatman GF/C filter paper 25 mm circle was from Whatman (UK), sodium dodecyl sulfate. N,N,N'-tetramethylthylene diamine (TEMED), ammonium persulfate (APS) were from USB (UK).

The goat anti-rabbit IgG alkaline phosphatase (AP) conjugated secondary antibody was from Bangalore Genei, Bangalore. The polyclonal antihistidine H3 antibody-ChIP grade (ab1791) abcam was a gift from Dr. Shubho Chaudhuri, Bose Institute, Kolkata. The enzymes RNase-free DNase I, DNase-free RNase I and restriction enzymes like BamHI, EcoRI were obtained from Fermentas and Exprime Taq DNA polymerase was from Genet Bio, Korea. Radiosotope like α-32P-dCTP (specific activity 4000Ci/mMol) was procured from Jonaki, Hyderabad (BRIT, India). Polyclonal antibody (purified IgG) generated against rat DNA Pol β was a generous gift from Dr. S H Wilson and Dr. R Prasad, NIEHS (National Institute of Environmental Health Sciences), USA.

#### Plant material

Seeds of Oryza sativa cv. IR 29, IR 8 were collected from Chinsurah Rice Research Institute,
Isoalation of buffer soluble protein and partial purification of protein

Seeds were surface-sterilized with 0.1% mercuric chloride and imibed overnight (16 h). Next day, seeds were spread over sterile gauge cloth soaked in autoclaved water and kept in dark at 37°C for germination. Total buffer soluble protein was isolated from the whole dry seeds, seeds imibed in water for 16 h and from the 12, 24, 36 and 48 h of germinated seeds as described later. To find out the effect of 250 mM NaCl and 20%PEG-6000 treatment, the 4-day-old germinated seedlings were treated with autoclaved double distilled water alone (control) or with salt or PEG for 4 h and 8 h. Samples were harvested and used in subsequent experiments.

Partial purification of DNA polymerase was done at 4°C following the protocol of Kumar et al with some modifications. Tissue (5 g) was homogenised in chilled mortar-pestle with 3 vols of ice cold protein buffer A (50 mM Tris–HCl, pH 7.5, 10 mM MgCl₂, 250 mM sucrose, 1 mM 2-mercaptoethanol, 0.1 mM EDTA, and 1 mM PMSF). Homogenate was centrifuged at 10000 g for 10 min at 4°C and supernatant was considered as S₀ fraction which was precipitated with ammonium sulphate to reach 70% saturation. Pellet was resuspended with 1 ml of protein isolation buffer and dialyzed against 100 vol of dialysis buffer B (50 mM Tris–HCl, pH 7.5, 1 mM 2-mercaptoethanol, 0.1 mM EDTA, 20% glycerol and 1 mM PMSF) and was estimated according to Bradford method. For further purification through DEAE-Sephacel column chromatography, 7.5 mg of protein was allowed to bind with 4 ml of equilibrated DEAE-Sephacel and poured into the column (2.1 x 5 cm). Unbound protein was washed with buffer B and the bound proteins were eluted by step elution with 0.3 M KCl. DEAE-Sephacel eluted protein was considered as partial purified protein.

Western blot analysis

20 µg of 70% ammonium sulphate cut and dialyzed fractions were separated through standard 10% SDS-PAGE along with standard molecular weight markers. Proteins were electro-transferred to PVDF membrane (GE Amersham Pharmacia, USA) using Mini VE vertical electrophoresis system (Amersham Biosciences, USA) by essentially following the manufacturer’s instructions. The blots were processed by affinity purified polyclonal anti-sera (purified IgG fraction) developed against rat DNA pol β or polyclonal antihistone H3 antibody (1:5000 dilution in TBST), followed by alkaline phosphatase (AP) conjugated goat anti IgG secondary antibody. Primary antibody recognized band on membrane was revealed by following the enzymatic assay of AP for colour development. The densitometric value of pol λ bands were divided by the densitometric value of histone bands and multiplied by 100 to produce the normalized value in percentage. Histograms were made for IR8, IR29 and Nonabokra samples.

In vitro DNA polymerase assay

In vitro DNA polymerase assay was done according to Sarkar et al with some modifications. DNA polymerase activity was measured by the incorporation of α-32P-dCMP (deoxyctydine monophosphate) using activated calf thymus DNA as template primer, followed by precipitation in 10% TCA (trichloroacetic acid) and counting the radioactivity on glass fibre filter. The incorporation was measured by Liquid scintillation counter (Perkin-Elmer Tri-carb-2800TR) after drying under heat lamp.

In-gel activity assay of rice DNA polymerase λ

To determine the DNA polymerase active polypeptide, in-gel activity analysis was carried out using 2 µg of partially purified enzyme as we did previously in Sarkar et al using the protocol by Blank et al with little modifications in the absence or presence of inhibitor (200-fold molar excess of dCTP over dCTP).

Genomic PCR to amplify the upstream of DNA pol λ gene, their cloning, sequencing and comparison within salt sensitive and salt tolerant rice cultivars

Genomic DNA was isolated from the plume of IR 29 and Nonabokra germinated seedlings by cetyl trimethyl ammonium bromide (CTAB) method. Genomic PCR was done to amplify the upstream of DNA pol λ using the primers OsPol P3 (forward 5'-GTTGGATCCAGTCCATGGAACTCCCG-3') and OsPol P3 (reverse 5'-GATCAAGCTTTTGCCTCACAAGCATTAAACC-3'), respectively (underlined regions are restriction sites of HindIII and BamHI, respectively). PCR was performed for 30 cycles at 53°C as annealing temperature.

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After PCR amplification, product was purified by QIAquick nucleotide removal kit (Qiagen, USA). Purified PCR product was ligated with pBSKS in the ratio vector: insert of 1:3 for efficient ligation at BamHI and HindIII site. Ligated products were transformed to competent E. coli strain DH5α (Clontech, USA) cells. Transformed cells were selected on LB-agar plate containing ampicillin at 100 µg/ml concentration, with 40 µl of X-gal (20 mg/ml) and 7 µl of 100 mM IPTG solution. White colonies were picked up randomly and from overnight culture the recombinant plasmids were isolated. The recombinant clones were selected by restriction digestion and sequenced using M13 forward (Catalogue no. #S0100) and M13 reverse (Catalogue no. #S0101) primers according to standard manufacturer protocol of Applied Biosystem, USA. Using their chemicals, the sequencing was done in ABI PRISM 310 Genetic analyzer.

Results and Discussion

Uchiyama et al. have first shown the enhancement of pol λ gene expression at transcript level in rice shoot tips and anthers. Like DNA pol λ, mitochondrial DNA pol γ can also be strongly inhibited by ddNTP. Moreover, results presented here showed the synthesis of newly synthesized DNA band in absence of ddNTP, whereas no such band appeared in presence of ddNTP.

Detection of DNA polymerase λ during rice seed germination

Seed germination is a complex physiological phenomenon involving many genes and they are regulated by environmental factors. It is considered to be the most critical phase in life cycle of plants. After 12-24 h of imbibition, sprouting occurs, where root emerges first from embryo and then shoot. During imbibitions, quiescent dry seeds resume metabolic activity with enzymes and components already present in them. Rehydration allows initiation of protein synthesis initially with stored components and later on with newly synthesized ribosomes and enzymes. Presence of stored mRNA is universal in plant species which plays an important role in early protein synthesis using existing ribosomes in the seeds. After imbibition, two discrete phases of DNA synthesis occur in seeds. In first phase, DNA damage repair occurs that produces intact DNA and in second phase DNA replication occurs. Total buffer soluble protein was isolated from dry, 16 h imbibed seeds and 12 h, 24 h, 36 h and 48 h water-soaked seedlings.

Fig. 1 shows photographs of the dry seeds, seeds imbibed for 16 h and germinating seeds at different times.

In vitro DNA polymerase assay with total protein showed incorporation of a low level of radio-labelled nucleotide in activated DNA (template: primer). Due to low abundance of the enzyme, partial purification was done by precipitation with 70% ammonium sulphate and then through DEAE-Sephacel column chromatography. Fig. 2A shows the result of DNA polymerase assay with partial purified protein isolated from dry, imbibed and different stages after imbibition in presence and absence of the inhibitor ddCTP. The activity of ddNTP sensitive DNA pol λ was absent in dry seeds, but was found to be increased significantly after imbibition and 48 h of germination.

To determine the presence of DNA pol λ, Western blot analysis was done with the antibody made against rat DNA pol β. Initially, Western blot with antihistone H3 antibody was done as H3 protein is a housekeeping protein, thus detecting its endogenous level. The Western blot analysis showed that though 67 kDa band was hardly visible in dry seeds, the protein band was detectable after imbibition of 16 h, but decreased during germination and after 48 h the level was enhanced to the same level as after imbibition. Enhancement was found to be 5-fold as detected from Western blot analysis (Fig. 2B). The increase of single polypeptide DNA polymerase activity after imbibition of seeds corroborated previous observation that in the first phase of DNA synthesis damage repairing of stored DNA occurs. Earlier, expression of DNA pol λ at protein level is
reported after imbibition in seeds stored for few years having higher DNA damage. Thus, DNA polymerase assay result was supported by both Western blot results and in-gel activity assay. The higher expression of the enzyme during germination stages of *O. sativa* probably helps the plant to protect DNA from damage and allows successful germination of seeds. During early seedling stage, enhanced DNA polymerase λ found in *Arabidopsis thaliana* probably provides protection against DNA damage.

By In-gel activity assay, DNA polymerase activity was detected due to the incorporation of α-32P-dCTP into the newly synthesized DNA strand using activated DNA as template: primer. In-gel activity assay was done with the partially purified protein isolated from the dry seeds and germinated stages. Equal amount of partially purified protein (2 µg) was loaded along with 1 U of Klenow enzyme as positive control. Figure 3 shows presence of very low or undetectable level of active DNA pol λ in dry seed. Less intense bands were found in 12, 24 and 36 h during germination, but after 48 h, the level of DNA pol λ protein showed higher incorporation of α-32P dCTP. Figure S1 († for details see footnote) shows the Coomassie blue stained 10% SDS-PAGE of partially purified protein. The result matched very well with the Western blot and in-solution DNA pol λ activity analysis.

All the results confirmed the presence of DNA pol λ enzyme during the early seed germination stages in *Oryza sativa* indica rice cultivar IR 8. Previously we have shown the expression of DNA pol λ protein (DNA pol β) from rice seeds germination steps and its involvement in BER.

**Presence of cis-acting elements in the upstream of DNA pol λ gene**

Using the plant promoter database (japonica cultivar), the 498 bp upstream region was identified before the 3' end of the previous gene Os06g0237300 (similar to LIM domain protein WLIM-1) on chromosome number 6 (Figure S2A. †For details see footnote). Earlier studies have revealed that cis-acting regulatory elements control various biological processes such as response to hormones, abiotic stress and developmental processes acting as important
molecular switches. Thus, we analyzed the pol L upstream for the presence of different cis-acting elements. An in silico analysis using plantCARE database revealed the presence of number of cis-elements associated with various environmental signals (Table 1) like anaerobic responsive element (ARE), many different LREs (light responsive elements) like Box4, C-box, SP1 and abiotic stress inducible cis-acting elements like DRE (dehydration responsive element) and HSE (heat shock element). SP1 is believed to be associated with light response in plants. Dai et al. have reported that overexpression of a Myb gene containing R1R2R3 domain Myb transcription factor increases tolerance to freezing, drought and salt stress in transgenic Arabidopsis. Since Myb gene is inducible by salinity stress andABA, it could be regulated by salinity or dehydration stress. Only single copy of the DRE was found in IR 29, whereas two copies in Nonabokra. The cis-acting element DRE is reported to be involved in dehydration, low-temperature and salt stress situation, HSE in heat stress responsiveness and Myb binding site in drought inducibility.

Inducible expression of DNA pol λ enzyme in the plumules of 4-day-old seedlings in response to salinity and dehydration stress

The DNA polymerase activity was measured from the 4-day-old seedlings treated with NaCl (250 mM) or 20% PEG-6000 for 4 h and 8 h in absence or presence of the inhibitor ddCTP for DNA pol λ (Fig. 4A). Moreover, comparison was made within the protein extracts prepared from the plumules of control and NaCl or PEG-treated seedlings of IR 29 and Nonabokra. The DNA pol λ activity was significantly increased by 4-7 folds during NaCl stress and to 3-fold by dehydration in IR 29; however, in Nonabokra, increase was less as compared to IR 29. Both salinity and dehydration stresses enhanced the protein level by 2.5 to 3.5-fold and 2 to 2.5-fold, respectively. It was clear that salinity stress induced the activity of DNA pol λ more significantly than 20% PEG-6000 treatment. In addition, the level of DNA pol λ protein was higher in IR 29 than in Nonabokra.

Western blot analysis with equal amount of protein from all the samples showed equal band intensity around 17 kDa with antihistone H3 antibody (Fig. 4C). The protein band developed against DNA pol β antibody was clearly visible in plumules of both IR 29 and Nonabokra only after NaCl or dehydration stress (Fig. 4B). In both cases, the antibody recognized the 67 kDa protein only after NaCl or dehydration stress. The induction was very prominent, 5-folds more after 8 h of salinity stress and 3-folds after 8 h of dehydration stress. Interestingly, increase was more in IR 29 than in Nonabokra. The data matched with the in-solution DNA pol λ activity analysis (Fig. 4A). In Nonabokra plumules, the increase was ~ 3-fold by NaCl stress and ~ 2-fold by 20% PEG-6000 treatment. Figure S3 (For details see footnote) shows the Coomassie blue stained gel of partially purified protein samples of 4-day-old germinated rice seedlings from IR 29 (A) and Nonabokra (B).

The activity gel done (Fig. 5) with DEAE-sephacel purified protein samples from both NaCl and PEG6000 treated samples showed that ddNTP sensitive DNA pol λ activity was enhanced more than 5 to 8-folds after treatment with NaCl and 3 to 4.5-folds by dehydration in IR 29.

DNA pol β (38 kDa) identified from human genome is absent in plants. DNA sequence homology of pol β with pol λ has shown high degree of

<table>
<thead>
<tr>
<th>Name of the element</th>
<th>Sequence</th>
<th>Function as described in plantCARE</th>
<th>IR-29</th>
<th>Nonabokra</th>
</tr>
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<tbody>
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<td>ARE</td>
<td>TGGTTT</td>
<td>cis-regulatory element essential for the anaerobic induction</td>
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<td>1</td>
</tr>
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<td>DRE</td>
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<td>AAAAAATTC</td>
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<td>1</td>
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<td>Sp1</td>
<td>TATCCCCA</td>
<td>Light responsive element</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 1—Presence of different cis-acting elements identified from the 498 bp upstream of pol λ gene of salt sensitive IR 29 and salt tolerant Nonabokra cultivars by using the plantCARE database

[Since the upstream of the 498 bp region of DNA pol λ gene, presence of another coding sequence (Os06g0237300) has been detected, it was considered that the DNA pol λ gene was under the control of 498bp upstream. Presence of DRE (Dehydration Responsive Element), HSE (Heat Shock Element) and MBS (Myb-binding site) were considered to play in the expression of pol λ gene in response to dehydration, heat and salinity stress]
homology at the C-terminal end of pol $\lambda$ which includes 5' deoxyribose phosphate lyase activity required to complement the DNA synthesis step associated with base excision repair\textsuperscript{6,37}. Recent study on repairing of UV-induced DNA damage via nucleotide excision repair pathway in *A. thaliana* suggests involvement of DNA pol $\lambda$ (AtPol $\lambda$). It has been detected in repairing double strand breaks induced by high salinity and DNA cross linking agent via the non-homologous end joining (NHEJ) pathway. The upregulation of AtPol $\lambda$ gene expression/transcriptional upregulation is also reported in response to abiotic e.g.

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**Fig. 4**—Detection of DNA pol $\lambda$ activity in absence or presence of ddNTP from the plumule of 4-day-old seedlings of IR 29 (i) and Nonabokra (ii) cv of indica rice (Fig. 4A) and by Western blot analysis using Pol $\beta$ antibody (Fig. 4B) and antihistone H3 antibody (Fig. 4C) treated with none (control) or 250 mM NaCl or 20% PEG\textsubscript{6000} for 4 h and 8 h [Ctrl = Control, N4 = 250 mM NaCl stress for 4 h, N8 = 250 mM NaCl stress for 8 h, P4 = 20% PEG\textsubscript{6000} stress for 4 h, P8 = 20% PEG\textsubscript{6000} stress for 8 h]. Photograph of IR-29 (left) and Nonabokra (right) are shown here.

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**Fig. 5**—Activity gel analysis showing enhancement of DNA pol $\lambda$ activity from the plumule of 4-day-old seedlings of IR 29 and Nonabokra in response to salinity stress and dehydration for 4 h and 8 h without ddCTP. (A) and (B) were without or with ddCTP respectively (C) shows the relative activity of (A) [Abbreviations as described earlier]
salinity and genotoxic stresses with DNA cross-linking agent mytomycin C\(^{38}\).

The results of this study clearly showed that both salinity stress and dehydration enhanced the protein level and enzyme activity of DNA pol \(\lambda\). The gradual increase of AtPol \(\lambda\) transcript level is reported in 7-day-old wild type Arabidopsis seedling exposed to increasing concentration of NaCl from 0 to 200 mM for 8 h. Here, we showed the increase in pol \(\lambda\) protein level and also its activity by Western blot analysis and activity gel analysis. Increase of DNA pol \(\lambda\) was about 5-fold in IR29 and 3-fold in Nonabokra after 8 h of 250 mM salinity stress, while for dehydration stress increase was less than salinity stress, but more than 3-fold in IR29 and 2-fold in Nonabokra after 8 h of 20% PEG-treated plants. Activity gel (Fig. 5) of partially purified protein showed higher increase in DNA pol \(\lambda\) activity in IR29 than Nonabokra in response to salinity and dehydration stress.

It is suggested that environmental stress enhances phytohormone e.g. abscisic acid which triggers the signaling process both locally and systemically in response to abiotic stress. This may induce gene modulation by chromatin remodeling and then by DNA repair of the genome which brings transcriptional stability which finally help the plants to adapt against the environmental stresses such as UV, salinity, dehydration or temperature stress\(^{39}\).

In conclusion, the present study demonstrated that pol \(\lambda\) protein was detectable in seeds only after imbibition and up to 3-days of germination, but the protein level was induced after 4-days, when subjected to abiotic stresses like high salinity or dehydration stresses. The \textit{cis}-acting elements detected from the 5' upstream region of pol \(\lambda\) gene in \textit{O. sativa} showed the presence of DRE, HSE and Myb binding site. All these sites are well-known for inducing the gene during the abiotic stress situation. This is the first report showing that the level of pol \(\lambda\) enzyme or the protein is enhanced during early stages of seed germination and salinity or dehydration stress in plumules of both salt-sensitive and salt-tolerant rice cultivars.

**Acknowledgement**

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**References**

14 http://irri.org/, accessed on September 10, 2013
15 http://www.cssri.org/, accessed on September 10, 2013
16 http://crri.nic.in, accessed on September 10, 2013