CHAPTER-5
Summary & Conclusions
SUMMARY AND CONCLUSION

In the cell DNA damage can occur due to several reasons like ROS, metabolic by-products and during DNA replication. The damage to the DNA if unrepaired could lead to DNA lesions, mutations, stalling of DNA replication and transcriptional complexes, etc. thereby inhibiting the growth and development of plants. In order to maintain the integrity of genome and preventing the above mentioned issues the DNA repair enzymes play critical role by repairing the damaged DNA and preventing the occurrence and passage of mutations to the next generation (Waterworth et al., 2015). The study shown here is one of the first reports of its kind where the total expression level and activity of DNA Polymerase \( \lambda \) is measured throughout the life cycle in rice. It will further help us to understand the nature and mechanism by which the X-family DNA Polymerase \( \lambda \) works during different developmental stages in rice, one of the most important cereal crops.

Abiotic stresses like salinity, drought and heat induce DNA damage both in plants and animals cells. We choose the early vegetative stages of 4 day old, 8 day old and 12 day old rice seedlings from three different rice cultivars to study the effect of different abiotic stresses. The \textit{in vitro} DNA polymerase assay using activated calf thymus DNA as standardized before (Sanath Kumar \textit{et al.}, 1996, Sarkar \textit{et al.}, 2004, Roy \textit{et al.}, 2007) was routinely performed to monitor the enzyme activity in rice plant biology. By this study there will also be a prospect to utilize the gained information and use it for the betterment of yield and quality of the indica rice cultivars in the long run.
From the extensive study of rice DNA Polymerase λ in three different indica rice cultivars, following conclusions could be drawn:

- A single copy of DNA Polymerase λ gene is present in the plant system, suggesting its importance as DNA Polymerase λ is reported to be the only X-family DNA polymerase. The ~45 kDa purified recombinant DNA Pol λ missing the N-terminal BRCT domain was found to have DNA polymerase activity and was sensitive to dideoxynucleotidetriphosphate. The protein was also analysed by in-gel activity and was detected by western blot analysis. Activity gel analysis revealed that the recombinant DNA Polymerase λ of *Oryza sativa* carried out DNA synthesis and was sensitive to ddNTP, similar to the positive control (Klenow) that was known to be a highly processive enzyme.

- Negligible gene expression and activity is found in dry seeds while enhancement of gene expression and the 67kDa single polypeptide enzyme DNA polymerase λ activity has been found after imbibition of dry seeds. The enzyme activity was detectable during the early seedling stages of germination. The activity again decreases and is negligible with the rice plant maturation. DNA polymerase assay and western blot analysis also showed the enhancement of the enzyme immediately after imbibition and during early stages of seed germination as compared to other stages of plant development suggesting the importance of DNA Polymerase λ during seed germination of rice.

- DNA Polymerase λ activity and gene expression was found to be enhanced by 8-9 folds during microspore formation in anthers from the three indica rice cultivars suggesting its role in DNA replication and/or repair in both meristematic and meiotic tissues.
During seed maturation, activity and gene expression of DNA Pol λ was comparatively high in the milky stage of seed development, gradually decreases in young seeds and is negligible in mature seeds. As in mature seeds, metabolism is reduced to very low levels and embryo becomes quiescent thus chances of damage reduces, the capacity for repair also reduces and thus the level of DNA Pol λ was undetectable from mature seeds.

A 498 bp long intergenic region upstream of the coding sequence of POLL (OSJNBA0068B06.5) was identified because of the proximity of the upstream gene (OSJNBA0068B06.6).

An in silico analysis of the 498 bp OsPolL promoter revealed the presence of a number of cis–acting elements associated with various environmental signals like many different light responsive elements, dehydration responsive elements, heat shock elements, salinity and temperature responsive.

To study the role of salinity, drought and heat for regulation of Pol λ, the activity was measured from the 4-day-old, 8-day-old and 12-day-old seedlings treated with 250mM NaCl or 20% PEG-6000 or heat (42°C) for 4 hrs and 8 hrs in absence or presence of the inhibitor ddCTP for DNA Pol λ. The study shows that the activity and the expression were found to be effected by all three treatments but salinity stress induced the activity of DNA Pol λ more significantly than 20% PEG-6000 or heat treatment. In addition the fold change of Pol λ was lowest in case of N22 cultivar while the expression of Polλ in Nonabokra was found to be at intermediate level as compared to IR29 and N22. Thus, Pol λ acts as an important component of nuclear DNA damage repairing in plant genome and a stress tolerance trait is associated with Pol λ function.
- Bioinformatic study of the ten selected proteins from *Oryza sativa* containing BRCT domain shows that all BRCT domains were highly homologous and were tightly clustered together as visible from their evolutionary comparison. DNA polymerase lambda was predicted to interact with DNA ligase I, Proliferating Cell Nuclear Antigen (PCNA), ATP dependant DNA ligase, ATP dependant DNA helicase, Proteasome subunit, different protease regulatory subunits, Adhesion regulating molecules, etc. through their BRCT domain. Predicted interactions of the other selected BRCT domain containing proteins was observed *in silico* with various crucial regulatory partners, which govern the stability and activity of the cell.

The experiments were designed in a way to get information on the expression of Pol λ gene and its protein by using many well-known techniques. The results presented here clearly show the presence of the transcripts or the activity of Pol λ protein during different times of rice plant growth and differentiation and also was found to be enhanced in response to abiotic stress like salinity, dehydration and high temperature in the three different indica rice cultivars varying in abiotic stress response and yield. Since the BRCT domain present in Pol λ protein is known to be involved in dimerization and appropriate function, bioinformatics analysis have identified ten different BRCT domain containing proteins from rice and their probable interactions.

Thus, from the comparative biochemical and molecular analysis the results presented here provide novel information about rice DNA Pol λ and gives a clear scenario about this enzyme in indica rice cultivars- IR29, Nonabokra and N22 suggesting that high yielding, abiotic stress sensitive IR29 rice plants were found to be better in DNA Pol λ gene and protein expression.