6. Summary and Conclusions

In the present study, the designed dominant negative proteins have been used to study and regulate the redundant behavior of B-ZIP transcription factors involved in seed maturation. Keeping in view the objectives, a dominant negative protein A-ZIP53 and four different derivatives of A-ZIP53 were designed and their efficacy were shown in vitro. A-ZIP53 transgenic plants were generated. A-ZIP53 transgenic Arabidopsis demonstrated the efficacy of our DN in vivo. These designed proteins can be used to regulate the DNA binding of target B-ZIP proteins through preferential heterodimerization.

Important finding of present study are as follows:

1. Seed Maturation is a combinatorial effect of the B-ZIP53, B-ZIP25, and B-ZIP10. We showed the dimerization of these B-ZIP TFs is DNA dependent. Circular dichroism and ESI-MS confirmed the importance of DNA in the heterodimer formation.

2. Replacement of the basic DNA binding domain of the B-ZIP53 with the designed acidic extension form a novel dominant negative protein A-ZIP53. The designed dominant negative protein was used to regulate heterodimerization of target B-ZIP transcription factors.

3. Circular dichroism and gel mobility shift assay showed the A-ZIP53 heterodimerizes with the B-ZIP53 and its heterodimerizing partners B-ZIP10 and B-ZIP25. The binding of A-ZIP53 is highly specific towards target B-ZIPs. It forms very stable heterodimer with B-ZIP53, B-ZIP10, and B-ZIP25. It did not heterodimerize with B-ZIP72 and B-ZIP39, two B-ZIPs expressed during seed maturation and known to play prominent role in regulation of seed-specific genes.

4. Four different derivatives of A-ZIP53 were designed by site directed mutagenesis with varying homo- and heterodimer stabilities. The derivatives of the A-ZIP53 have different affinities towards B-ZIP53, B-ZIP10, and B-ZIP25.

5. The A-ZIP53 (A→E and N→A) has strong affinities for B-ZIP53, B-ZIP10, and B-ZIP25 compared to the other derivatives.
6. Transient transfection assay using *Arabidopsis* protoplast confirmed the efficacy of A-ZIP53 *in vivo*. The GUS/NAN assay showed that A-ZIP53 inhibits the DNA binding activity of target B-ZIP TFs.

7. The transgenic *Arabidopsis* expressing A-ZIP53 showed range of phenotypes. The varying phenotypes in different lines correlate with the differential expression of the A-ZIP53.

8. Transcriptome analysis of immature siliques and seeds of transgenic *Arabidopsis* showed the lower expression of genes responsible for the seed development and maturation. Genes were shortlisted which have DNA binding site for target B-ZIP TFs in their promoter region. The validation of the down regulated target genes were confirmed by the qRT-PCR.

9. Physiological parameters of A-ZIP53 expressing transgenics were studied that showed delayed growth and smaller rosette diameter. Reproductive phase parameters of transgenics are analyzed that have smaller flower size, shorter siliques length, lesser number of seed within a siliqua and abnormal and small sized seeds.

10. Germination assay showed the seeds of the A-ZIP53 expressing transgenic lines were non-viable and did not germinated on the plain M.S. media.

11. Total protein from the immature siliqua and seeds of the A-ZIP53 expressing transgenics were subjected to the immunoprecipitation followed by mass spectrometry. Eight different heterodimerizing B-ZIP proteins (B-ZIP14, B-ZIP17, B-ZIP19, B-ZIP23, B-ZIP29, B-ZIP33, B-ZIP34, and B-ZIP69) were identified that are putative dimerizing partners of the B-ZIP53 and its heterodimerizing partners.

The present findings clearly demonstrate that designed dominant negative proteins can used to regulate the DNA binding activity of B-ZIP TFs and their heterodimerizing partners. Dominant negative protein is a unique tool that may be used to address the redundant behavior of the target proteins. This strategy is unique and it is not possible by other loss of function mutation techniques including RNA interference and CRISPR/Cas. It is shown that designed A-ZIP53 is found to be specific for B-ZIP53 and its closely related B-ZIPS TFs. It is found to be ineffective in forming heterodimer with B-ZIP39 and B-ZIP72. In addition, we identified hitherto unknown partners of B-ZIP53 namely, B-ZIP14, B-ZIP17, B-
ZIP19, B-ZIP23, B-ZIP29, B-ZIP33, B-ZIP34, and B-ZIP69 that may play role in seed development and maturation. In future homologs of these seed-specific B-ZIP TFs will be studied in fruit crops like kinnow and guava. We hope that this study will help in the development of seedless fruits.