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Title of the thesis: Transcript analysis and molecular mapping of genes from chickpea (*Cicer arietinum* L.)

Abstract

In the present study two important legume functions were targeted i.e. seed development and heavy metal (Cd) stress. Section I describes studies based on seed development which included generation and analysis of ESTs by constructing a cDNA library from seed tissue, annotation and expression profiling of a set of genes in various stages of seed development and utilization of the seed ESTs for development of genic molecular markers for construction of a linkage map of chickpea. Section II covers studies based on heavy metal stress (Cd) which includes generation and analysis of ESTs by constructing a cDNA library from root-tip tissue of chickpea seedlings under Cd stress, identification and expression analysis of genes from different functional categories and utilization of ESTs for development of genic molecular markers. The present thesis comprises of three chapters that are summarized below:

I

Chapter 1: Generation of ESTs from chickpea seeds and expression analysis at various stages of development

A cDNA library was constructed from 10 DAA immature seeds of chickpea ICCV2. Single pass sequencing yielded a total of 1343 good quality ESTs which were assembled into 685 unigenes. Of the 685 unigenes, 87.2% produced blast hits and 12.8% did not find any similarity with publically available sequences. A reverse northern experiment was performed with 276 cDNA clones and their expression analysis was studied in four stages of seed development (10, 20, 30, 40DAA). An expression map was generated which revealed that a relatively higher number of genes were expressed at early stages (10 and 20DAA) than the later stages (30 and 40DAA) of seed development. 55.4% (153) genes were found to be differentially expressed in at least one of the stages of seed development, as compared to other stages. To further confirm the macroarray gene expression data, a set of 28 genes showing >1.5 fold change in their expression values were selected for real-time PCR in eight experimental tissues including flower bud, leaf, root and 5 seed development stages (5, 10, 20, 30 and 40DAA). All tested genes were validated by qRT-PCR in their target stages at levels similar to those observed in macroarray.

Chapter 2: Development of EST-derived molecular markers in chickpea for construction of the linkage map of chickpea

The ESTs generated from 10DAA seed library were utilized for development of various types of molecular markers such as EST-SSRs, Potential Intron Polymorphisms (PIPs) and Expressed sequence tag polymorphisms (ESTPs). Total 260 genic molecular markers were developed, of which, 175 were found to be functional. Of these, 64 were found to be polymorphic between the parents, *C. arietinum* ICC4958 and *C. reticulatum* PI489777, of an inter-specific mapping population, and further genotyped in 129 RILs of mapping population. A linkage map was generated utilizing the genotyping data of 64 (this study) and 296 markers (previously mapped) at LOD 4.0 using JoinMap. 352 markers were mapped onto 8 linkage groups and spanned the total genetic length of 1595.6 cM of the chickpea genome with an average marker density of 4.53 cM.

II

Chapter 3: Generation and analysis of ESTs under Cd stress for identification of heavy metal stress responsive genes in chickpea

A cDNA library from chickpea root-rip tissue of 2 week old seedling under Cd stress was constructed. Total 1597 good quality ESTs were generated that assembled into 914 unigenes. Of the 914 unigenes, only 38.8% showed significant matches to putative genes and 61.2% did not show any hits. Relative quantification was performed with the genes encoding putative transcription factors and transporters from Cd stressed root-tip cDNA library. Five out of eight TFs corresponding to heat shock TF, WRKY TF, WRKY6, NAC domain containing protein and AP2 domain class TF were activated specifically in root tissue after 6hrs of Cd treatment, whereas 3 TFs namely Myb, homeobox protein, and another WRKY TF showed differential expression in stressed tissues in comparison to control tissues. Moreover, the generated ESTs were utilized for development of 83 genic molecular markers which included 34 EST-SSRs and 49 ITPs.