INTRODUCTION

REVIEW OF LITERATURE

General overview of chloroplast protein import
Translocation by the Toc/Tic pathway
Translocation of chloroplast preproteins from the cytosol to Outer Envelope Membrane (OEM)
Core components of the TOC complex
Toc159
Toc34
Toc75
Toc64
Toc12
Regulation of Protein Import at TOC Complex
Core components of the TIC complex
Tic110
Tic62
Tic55
Tic40
Tic32
Tic22
Tic21
Tic20
Regulation of Protein Import at TIC Complex
Redox Regulation
Calcium Regulation
Stromal processing peptidase
ROLE OF CHAPERONS IN PROTEIN IMPORT
Cpn60
Hsp70
Hsp93
INSERTION INTO THE OUTER MEMBRANE OF CHLOROPLAST
IMPORT INTO THE INTERMEMBRANE SPACE AND INSERTION INTO THE INNER MEMBRANE
PROTEIN IMPORT INVOLVING THE SECRETORY PATHWAY
PROTEIN IMPORT INTO THYLAKOIDS OF CHLOROPLAST
Targeting to the thylakoid membrane
Targeting to the thylakoid lumen

MATERIALS AND METHODS

Plant materials and growth condition
General sterilization procedures
Nutrient media
Chemicals
Recombinant DNA techniques for cloning and DNA analysis
Polymerase chain reaction
Setting up a restriction digest
Purification of DNA fragment from agarose gel
Preparation of competent cells and transformation
Isolation and purification of plasmid DNA
Spectrophotometric estimation of nucleic acid
PTGS Construct Preparation of AtTic22 (At4G33350)
Transformation of Agrobacterium
Antisense and sense Construct Preparation of AtCpn60α (At2g28000)
Expression of Gene Products
Overexpression of AtCpn60α in E. coli
Expressed of AtCpn60α mature Protein
Polyacrylamide gel electrophoresis of proteins (SDS-PAGE)
Staining with coomassie brilliant blue R 250 (CBB R 250)
Purification of Recombinant AtCpn60α protein
Western blot
Raising of polyclonal antibody
Arabidopsis transformation by vacuum infiltration
Plant growth procedure
Vacuum infiltration
Selection of Transgenic Plants on Kanamycin Plate
Analysis of transgenic plants
Preparation of plant genomic DNA for PCR analysis
PCR analysis of transgenic plants
Analysis of transcript levels
Isolation of total RNA by TRI reagent (Sigma) method
RNA gel
Northern hybridization
Preparation of probe by random primer labeling method
DNase I treatment of total RNA
Semi-quantative RT-PCR
Real-Time PCR
Identification of Homozygous Mutants through PCR
Transmission electron microscopy
Plant growth conditions for Tissue and Developmental studies
Light Treatment
Isolation of intact chloroplasts
Preparation of precursor proteins
Import/binding of precursor proteins
Stress treatment of plants
Chlorophyll and carotenoid estimation
Chlorophyll a fluorescence measurements

RESULTS

Functional genomics of Arabidopsis atTic22, a putative component of chloroplast protein import apparatus
Sequence alignment of atTic22
Dendogram
Kyte and Doolittle Hydropathic plot
Hopp and Woods Antigenecity Plot
Tissue specific expression of atTic22
Developmental expression of atTic22
Light-independent atTic22 expression
Post-translational gene silencing of atTic22 in Arabidopsis
Construction of plant transformation vector with AtTic22 PTGS construct. 86-87
Plant transformation 87
Confirmation of transgenic plants 88
PCR analysis 88
Semi-quantitative RT PCR analysis of the AtTic22ptgs lines 88
Northern Hybrizidation 89
Quantitative Real Time PCR Analysis of the WT and AtTic22ptgs lines 89
Western blot analysis of the AtTic22ptgs lines 89
Screening of Arabidopsis Homozygous atTic22 Mutant 89
Expression of genes encoding different components of the chloroplast protein import apparatus 90
Protein abundance of the components of the chloroplast protein import apparatus (Western Blot Analysis) 90-91
Phenotypic Analysis of WT, tic22 mutant and atTic22ptgs Plants 91
Pigment content of WT, tic22 mutant and atTic22ptgs Plants 91-92
Chloroplast Ultrastructure studies of WT, atTic22ptgs and tic22 mutant plants 92
Pulse amplitude modulated (PAM) chlorophyll a fluorescence measurements 92
Import of Preproteins in WT, atTic22ptgs and tic22 mutant plants 93
Analysis of WT, tic22 mutant and AtTic22ptgs plants under various abiotic stresses 94-98
Effect of Salt Stress on WT, tic22 mutant and atTic22ptgs plants 94-96
Effect of Heat stress in WT, tic22 mutant and atTic22ptgs plants 96-97
Effect of Chill stress in WT and tic22 mutant plants 97-98

Functional analysis of AtCpn60(α) subunit 98-111
  Sequence analysis 98
  Kyte and Doolittle Hydropathic plot 98
  Hopp and Woods Antigenecity Plot 99
  Developmental expression of atCpn60(α) 99
  Light-dependent atCpn60(α) expression 100
  Cloning of mature fragment of AtCpn60(α) 101
  Overexpression of AtCpn60(α) in E. coli 101
  Production of polyclonal antibodies against AtCpn60(α) 102
  Cloning of full length AtCpn60(α) cDNA from A. thaliana 102
  Overexpression and Downregulation of AtCpn60(α) in plants 102
  Cloning of AtCpn60(α) in sense and antisense orientations in plant binary vector 102
  Plant transformation 103
  Confirmation of transgenic plants 103
  PCR analysis 103
  Gene Expression of atCpn60(α) in WT and atCpn60(α)x lines 104
  Semi-quantitative RT PCR analysis 104
  Northern Blot analysis 104
  Western blot analysis 105
  Gene Expression of atCpn60(α) in WT and antiCpn60(α) lines 105
  Semi-quantitative RT PCR analysis 105
  Real Time PCR analysis 105
  Western blot analysis 105
  Phenotypic Analysis of WT, atCpn60(α)x-3 and antiCpn60(α)-2 106
  Pigment content of WT, atCpn60(α)x-3 and antiCpn60(α)-2 plants 106
  Expression of genes encoding different components of the chloroplast protein import apparatus. 106
  Protein abundance of the components of the chloroplast protein import apparatus 107
  Chloroplast Ultrastructure studies of WT, atCpn60(α)x-3 and antiCpn60(α)-2 plants 107
Pulse amplitude modulated (PAM) chlorophyll a fluorescence measurements 108
Import of Preproteins in WT, \textit{atCpn60(a)}x-3 and \textit{antiCpn60(a)}-2 plants 109
Analysis of WT, \textit{atCpn60(a)}x-3 and \textit{antiCpn60(a)}-2 plants under abiotic stresses 110
Effect of Heat stress in WT, \textit{atCpn60(a)}x-3 and \textit{antiCpn60(a)}-2 plants 110-111

**DISCUSSION**

Functional genomics of \textit{Arabidopsis atTic22},
a putative component of chloroplast protein import apparatus 113-117

Functional analysis of \textit{AtCpn60(a)} subunit 117-124

**SUMMARY**

125-129

**REFERENCES**

130-151