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A Variation of Gene expression of Protein Translocon at the Outer Envelope of Chloroplast: a brief overview

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Abstract

In plants, the chloroplast is the site for essential functions that are critical for many biosynthetic pathways. Due to the transfer of majority of its genes to the host nucleus during the endosymbiotic event, a host majority of the chloroplast proteins are nuclear encoded and post-translationally targeted into the chloroplast. Thus, protein trafficking into the chloroplast plays a pivotal role in regulation of chloroplast biogenesis. Among the various components of the translocon machinery, the translocon at the outer envelope of chloroplast (TOC) dictate the selectivity of the preproteins that have to be imported into the chloroplast for proper chloroplast function. Studies of the components of the TOC complex suggest that change in expression of the genes for TOC complex critically influence the chloroplast biogenesis and other related plant processes. Owing to the advancement in chloroplast biotechnology and increasing usage of chloroplast as a platform for crop improvement and production of biochemicals, it is of utmost importance to understand the expression pattern of the genes of the TOC complex. Using the publicly available gene expression database, we briefly discuss the expression pattern of the genes of TOC complex at different growth, development and stress conditions. An overview of the gene expression responses of the TOC complex will enhance our understanding of the chloroplast physiology which is fundamentals to chloroplast engineering for crop improvement and its industrial applications.

Keywords: Chloroplast biotechnology, protein import, TOC, expression, productivity, stress.

Chloroplast are specialized plant organelle that are site for photosynthesis, a process in which the solar energy is harvested and convert into usable chemical energy. Apart from photosynthesis, the chloroplast is also a site for various other essential plant processes, including biosynthesis of amino acids, nucleotides, lipids and hormones. They too play crucial role in nitrogen and sulphur assimilation [1-3]. During the evolution of chloroplast, thousands of the chloroplast genes were transferred to the host nuclear genome. Thus, although the present-day chloroplast contains ~2500-4000 proteins, its genome only encodes for ~100 proteins. To carry out the physiological process, chloroplast imports ~95% of the chloroplastic preproteins that are nuclear-encodes and post-translationally imported into the chloroplast [3, 4]. With growing

Translocon at the Outer envelope of Chloroplast (TOC)	
Gene	Accession no.
atTOC159	AT4G02510
atTOC132	AT2G16640
atTOC34	AT5G05000
atTOC33	AT1G02280
atTOC75-III	AT3G46740

Table 1. The genes and the accession number of the components of the translocon at the outer envelope of the chloroplast (TOC) that were used for transcript analysis

human population and increasing stress conditions, the need for crop improvement for higher productivity is of paramount importance to the plant biotechnologist. Chloroplast turns-up to be an excellent platform for the achieving high yield and stress resistance crop varieties. The chloroplast has also emerged to be a major platform in the industrial biotechnology field for the production of biochemicals. Both, nuclear-genome and chloroplast-genome transformation approaches are been extensively used to increase the abundance of chloroplastic proteins for achieving higher productivity and production of biochemicals in chloroplasts [5, 6].

The preproteins are targeted into the chloroplast through a hetero-oligomeric protein complexes, TOC-TIC complex, present on the outer and inner envelope of the chloroplast. The process of protein import into chloroplast has been maximally studied in the model plant, *Arabidopsis thaliana* [7]. Various research using biochemical and genetics approach had generated information that provides a significant understanding on the mechanistic details of the protein import into chloroplast [8]. In general, the nuclear encoded chloroplastic preproteins contain a N-terminal transit peptide that is recognized by receptor components of the TOC complex. The early steps of the preprotein targeting to the receptors is also aided by multiple cytosolic factors [9]. The receptors pass on the preprotein to the associated membrane channel protein on the outer envelope which thereafter is delivered into the chloroplast by concerted action of the TIC complex and other import associated factors [10]. The atTOC159- and atTOC34- family of proteins act as the primary receptors on the outer envelope of chloroplast in Arabidopsis, whereas atTOC75 form the channel protein [11]. The TOC complexes are organized in two primary forms: one comprising of atTOC159-atTOC33-atTOC75 (hereinafter referred to as TOC complex-I), primarily responsible for targeting photosynthetic preproteins, and the other consisting of atTOC132-atTOC34-atTOC75 (hereinafter

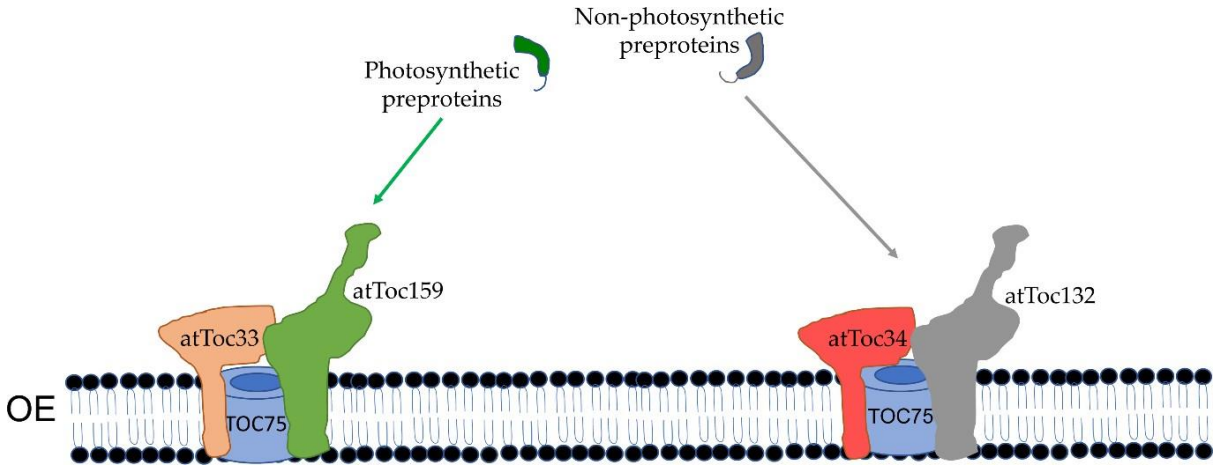

Figure 1. Working hypothesis of preprotein recognition and import through the translocon at the outer envelope of the chloroplast (TOC). The atToc159 associate with atToc33 to form the TOC complex (TOC complex-I) which predominantly recognize the photosynthetic preproteins. The atToc132 associate with atToc34 to form the alternate TOC complex (TOC complex-II) that primarily recognize the non-photosynthetic preproteins. Nevertheless, once the preproteins are recognized by the TOC receptors, they are transported through the outer envelope by a membrane channel protein, atToc75. OE: outer envelope of chloroplast.

referred to as TOC complex-II), thought to be responsible for targeting non-photosynthetic preproteins [8, 12] (Fig. 1). Among the multiple *atTOC75* genes, *atTOC75-III* encodes the atTOC75 member that form the membrane channel of the TOC complex (Baldwin et al, 2005) (Table 1).

Biochemical and genetic studies have revealed that change in the gene expression of the components of TOC complexes severely effect both photosynthetic and non-photosynthetic activities in the chloroplast [13-17]. The impairment has largely been contributed by the reduces import of the preprotein into the chloroplast as well as hinderance in the chloroplast-nuclear crosstalk. Additionally, the efficiency of various processes in chloroplast has also been suggested to be dependent on the age of the chloroplast [18]. The age-dependent regulation of chloroplast function was suggested to be due to the difference in the recognition of various groups of preprotein by the receptors at different developmental ages of the chloroplast. With diverse growth and developmental phases and constantly changing abiotic and biotic environment, the changes in the expression of the nuclear-encoded chloroplast proteins and their targeting to the chloroplast could also have a cumulative impact on the overall chloroplast

efficiency. A crucial step in this process is the specificity and differential affinity of the TOC receptors for various groups of preprotein under different conditions.

Owing to the critical role of the TOC complex in the chloroplast function, it becomes essential for biotechnologist to have a complete knowledge on the expression pattern of the genes of TOC complex. To broaden our understanding on the expression of the genes of the TOC complex, we utilized the expression database publicly available for *Arabidopsis thaliana* (GENEVESTIGATOR; www.genevestigator. com) to study the differential gene expression profiles of different members of the TOC complex [19] (Fig. 2). Tissue specific expression analysis suggest that the overall expression of *atTOC159*/*atTOC33* are comparatively higher than the expression pattern of *atTOC132*/*atTOC34*, thereby suggesting a higher abundance of TOC complex-I containing plastids in most of the tissue (Fig 2A). The *atTOC159* and *atTOC33* maximally expressed together in cotyledon, pistil, seed, juvenile leaf and shoot apex. They were minimally expressed together in stamen, nodes and roots. The higher expression in the cotyledon and juvenile leaves can be corelated with the higher photosynthetic activities in these tissues. In the same vein, their minimal expression in the roots may reflect their minimal requirement in the non-green plastids. The roots showed higher transcript abundance of *atTOC132*/*atTOC33*, corroborating the earlier proposed essential role of the *atTOC132*/*atTOC34* in the non-green plastids [13, 14]. Interestingly, a very the low abundance of the *atTOC159*/*atTOC33* genes were observed in the stamen whereas their expression was seen to be elevated in the pistil. A somewhat similar pattern of expression was also observed for the *atTOC132*/*atTOC34*. This may reflect specific role of the plastid activities in the flower development. The expression analysis suggests that *atTOC75-III* is expressed ubiquitously in most of the tissues (Fig. 2A).

Regarding the developmental expression pattern, the *atToc33* gene had maximal expression in all developmental stages analyzed as compared to all other genes of TOC complex (Fig. 2B). In the same vein, the *atTOC159*/*atTOC33* expression was higher than the *atTOC132*/*atTOC34*, suggesting that the TOC complex-I is more abundant than the TOC complex-II throughout all the developmental stages analyzed. The expression of *atTOC75-III* is almost similar

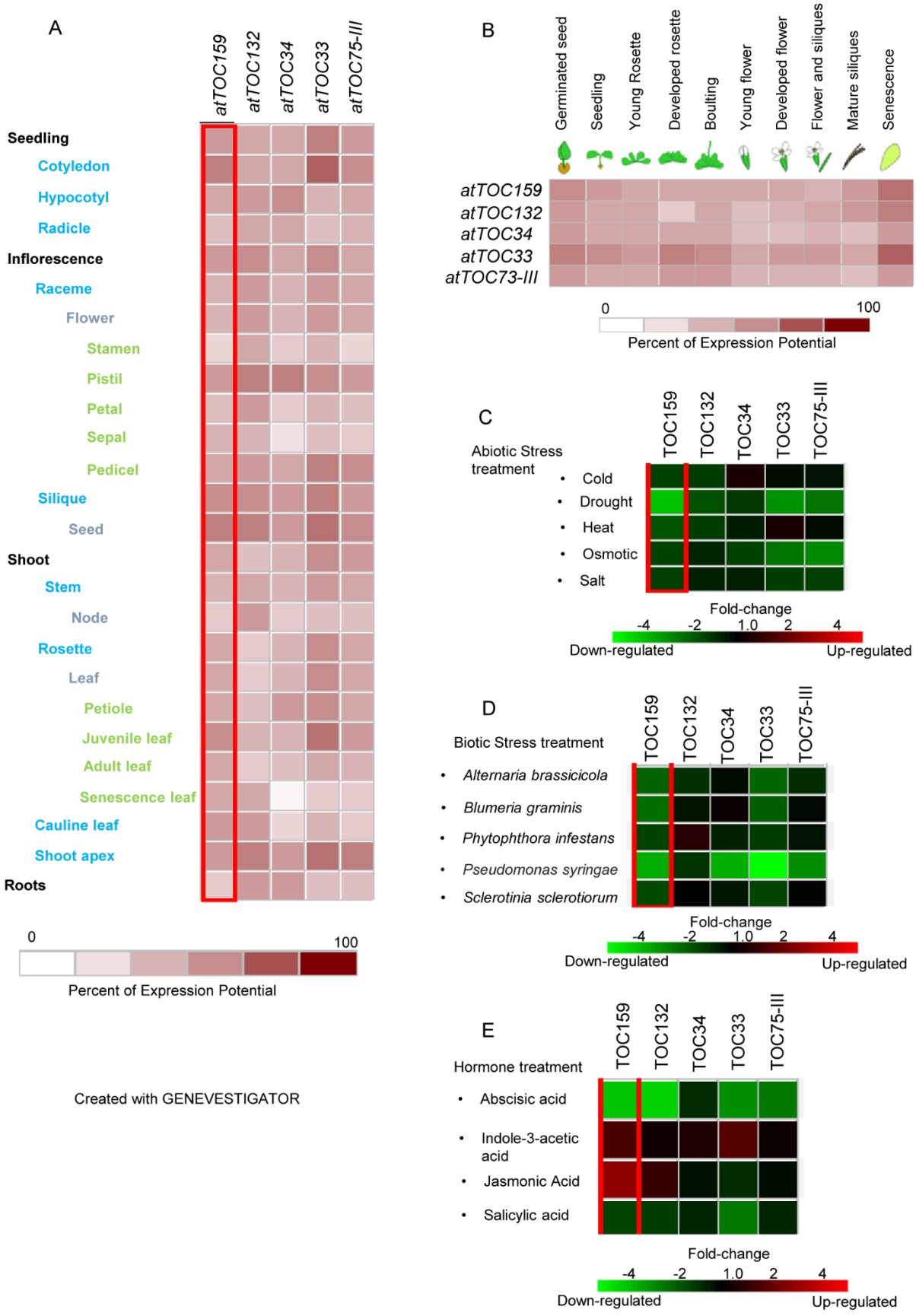
A
atTOC159
atTOC132
atTOC34
atTOC33
atTOC75-III
Seedling
Cotyledon
Hypocotyl
Radicle
Inflorescence
Raceme
Flower
Stamen
Pistil
Petal
Sepal
Pedicel
Silique
Seed
Shoot
Stem
Node
Rosette
Leaf
Petiole
Juvenile leaf
Adult leaf
Senescence leaf
Cauline leaf
Shoot apex
Roots
0
100
Percent of Expression Potential
Created with GENEVESTIGATOR
B
Germinated seed
Seedling
Young Rosette
Developed rosette
Boulting
Young flower
Developed flower
Flower and siliques
Mature siliques
Senescence
atTOC159
atTOC132
atTOC34
atTOC33
atTOC73-III
0
100
Percent of Expression Potential
C
TOC159
TOC132
TOC34
TOC33
TOC75-III
Abiotic Stress treatment
Cold
Drought
Heat
Osmotic
Salt
Fold-change
-4
-2
1.0
2
4
Down-regulated
Up-regulated
D
TOC159
TOC132
TOC34
TOC33
TOC75-III
Biotic Stress treatment
Alternaria brassicicola
Blumeria graminis
Phytophthora infestans
Pseudomonas syringae
Sclerotinia sclerotiorum
Fold-change
-4
-2
1.0
2
4
Down-regulated
Up-regulated
E
TOC159
TOC132
TOC34
TOC33
TOC75-III
Hormone treatment
Abscisic acid
Indole-3-acetic acid
Jasmonic Acid
Salicylic acid
Fold-change
-4
-2
1.0
2
4
Down-regulated
Up-regulated

Figure 2. Gene expression analysis of the components of the translocon at the outer envelope of chloroplast (TOC) in *Arabidopsis.* The *Arabidopsis TOC* genes were analyzed for organ-specific (A), developmental (B), abiotic (C) and biotic stress treatment (D) and hormone treatment (E) expression pattern using the GENEVESTIGATOR program. (A) Heat map analysis of *TOC* genes expression in different anatomy parts during development. (B) Heat map analysis of *TOC* genes expression during different stages of development. (C) Heat map analysis of *TOC* genes expression in response to abiotic stress treatment. The microarrays datasets used represent Genevestigator sets AT-00120, AT-00633, AT-00645 and AT-00684. (D) Heat map analysis of *TOC* genes expression in response to biotic stress treatment. The microarrays datasets used represent Genevestigator sets AT-00108, AT-00309, AT-00406, AT-00661 and AT-00681. (E) Heat map analysis of *TOC* genes expression in response to hormone. The microarrays datasets used represent Genevestigator sets AT-00320, AT-00433, AT-00655 and AT-00683. The intensity of red colour corresponds to the degree of gene expression increase, and the intensity of green colour corresponds to the degree of gene expression decrease in response to abiotic- (C), biotic stress (D) and hormone treatment (E). Black colouration denotes no difference in the gene expression between control and treatment conditions.

in all the developmental stages. The high fluctuating expression pattern of the receptor components compared to the membrane channel protein advocates a more regulatory role of the receptor components in the protein targeting to chloroplast.

Genevestigator analysis suggests a differential expression pattern of genes of TOC complex in response to various abiotic and biotic stresses (Fig. 2C, D). The expression of all the candidate genes were adversely affected by the abiotic stresses. A comparative analysis suggests that expression of the *atTOC159* is most severely impaired than other members of the TOC complex. Among the limited abiotic stress conditions analyzed, the drought stress appeared to have the most profound effect on the transcript abundance of the TOC complex. Interestingly, temperature stress, both heat and cold, seems to impair the expression of only Toc159- family of receptors, whereas the expression of *atTOC34*, *atTOC33* and *atTOC75-III* remained almost equivalent to control under those stress conditions. A downregulation in expression of *Toc159* have also been earlier reported in peas under high temperature stress conditions (Dutta et al. 2009). Overall, from the analysis of the expression pattern it appears that the *atTOC159*/*atTOC33* gene expression are more severely affected by abiotic stresses. Thus, reduction in the abundance of the TOC complex-I on chloroplast under abiotic stresses may be significantly contributing towards decrease in the photosynthetic efficiency under those conditions. Of the few biotic stresses analyzed, the genes for TOC complex exhibited a moderate downregulation in

response to pathogen challenges (Fig. 2D). The only exception to the above generalized observation was observed under the *P. syringae* treatment. The treatment of the bacteria, *P. syringae* resulted in a sever impartment in the expression of *atTOC159*, *atTOC34*, *atTOC33* and *atTOC75-III* genes. The change in abundance of genes of TOC complex following hormone treatment varied significantly depending on hormone type (Fig. 2E). A decrease in the expression of all the genes of TOC complex was observed following abscisic acid and salicylic acid treatment. On the contrary, *atTOC159* was found to be significantly upregulated following indol-3-acetic acid and jasmonic acid treatment. The variation in gene expression of the TOC complex in response to various hormone treatments suggest genes of TOC complex, and specifically *atTOC159*, to be few of the crucial target genes linking hormone signaling to numerous chloroplast functions. Overall, the diverse response of the genes of TOC complex hint towards presence of potential Cis-acting regulatory elements (CREs) on the promoters of these genes that may be associated with wide variety of physiological and biochemical responses, including responses to biotic, abiotic stresses, growth and developmental responses and responses to phytohormones.

In summary, the genes for the TOC receptors in Arabidopsis are highly responsive not only to the different growth and development phases but also to various environmental conditions. The fluctuation is predominantly at the receptor level with *atToc159* and *atToc33* genes showing maximum sensitivity to the changing plant phases or conditions. Thus, fluctuation in the abundance of the TOC complex-I appears to be a critical chloroplastic response to changing growth and environmental conditions. This variation in the TOC complex-I abundance can be directly linked to the downstream photosynthetic performance of the chloroplast. Nevertheless, the observed change in *atTOC132* and *atTOC34* at various plant phases and conditions analyzed also suggest that the efficiency of non-photosynthetic processes in chloroplast may also be readjusted and/or impaired under certain conditions. It has been suggested that both the receptors, Toc159 and TOC34, are limiting factors in the formation of TOC complex in peas [20]. The nuclear-transformation or chloroplast-transformation are the two approaches adapted by the biotechnologist for achieving higher chloroplastic performance. A highly efficient post-translational chloroplast targeting is an essential prerequisite for achieving the

desired traits in the nuclear-transformation approach. On the other hand, establishment and maintenance of efficient expression system inside the chloroplast is of paramount importance for maximal synthesis of protein in the chloroplast-transformation approach. Thus, both these approaches profoundly rely on the TOC complex for achieving optimum efficiency. Hence, the optimum expression of the genes for TOC complex are critical for achieving high chloroplast efficiency, i.e. effective expression of transgenes for obtaining high crop productivity as well as efficient production of the biomaterial. A knowledge of the gene expression responses of the components of TOC complex at different stages of plant growth as well as under different environmental conditions will greatly enhance our interpretation and/or predicting the overall chloroplast efficiency under diverse growth, development and stress conditions.

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