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Arabidopsis LTP12, A Homolog of SIP470, As a Key Player in Biotic and Abiotic Stress Response Signaling Pathway

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LTP12, A Homolog of Tobacco SIP470, as a Key Player in Abiotic Stress Signaling Pathway in Arabidopsis *thaliana*

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ABSTRACT

Lipid transfer proteins (LTPs) are a member of the pathogenesis-related protein family (PR-14) and are believed to participate in the defense mechanisms of plants. This study aims to characterize the function of a mutant ltp12 (AT3G51590) in Arabidopsis *thaliana*, which is a homologous lipid transfer protein to SIP470 from Nicotiana *tabacum*, known for its role in abiotic and biotic stress. Specifically, the research will analyze the mechanism of abiotic stress in both the ecotype Col-0 and ltp12, using stressors such as NaCl and Mannitol. Previous reports suggest that knockout lines of ltp12 display a defective growth phenotype and lower expression of systemic acquired resistance (SAR). By understanding the roles of both Col-0 and ltp12 in abiotic stress, this study provides a new perspective on addressing the problems of increasing drought and climate change. Additionally, the research will discuss the subcellular localization of ltp12.

INTRODUCTION

Since their emergence, land plants have had to survive in a challenging environment. Plants face many challenges from abiotic factors, which can cause significant damage to agriculture and the environment, resulting in reduced crop yields. Moreover, due to the sessile nature of plants, they must confront abiotic and biotic stresses and develop mechanisms to avoid or tolerate to survive and thrive. Therefore, understanding the role of lipid transfer protein (LTP) in coping with abiotic stresses such as drought, salinity and mannitol are widely carried out in model plants like Arabidopsis *thaliana.*

LTP is a multigene family occurring in several genus of plant families with diverse range of expressions. Arabidopsis *thaliana* was initially found to have 15 genes that met the strict criteria for being LTPs, but subsequent research has suggested that there may be up to 50 putative LTPs present. Although various roles have been suggested for LTPs, there is limited conclusive evidence regarding the specific function of individual isoforms. LTPs have been linked to several biological processes, such as responses to abiotic stress and pathogen defense, and they are recognized as PR proteins and classified as members of the PR-14 family. Studies suggest that several signal molecules, including abscisic acid, salicylic acid, ethylene, and methyl jasmonate, play a role in regulating the expression of LTP genes. Salicylic acid is crucial for plants to cope with stressors such as salinity, drought, and pathogen by mediating resistance (Kumar et al., 2014). Thus, understanding Arabidopsis LTP and its homolog such as SIP-470 (Tobacco) can provide insight on new pathways to plant defense mechanisms against abiotic and biotic stress.

MATERIALS AND METHODS

In this research, we grew Arabidopsis ltp12 (a knockout mutant) and the wild-type col-0 seeds on ½ MS media in a controlled environment. These seedlings were subjected to various stresses, e.g. NaCl (salinity) and Mannitol (osmotic) (50 mM, 100 mM, and 150 mM). The leaves from 3-weeks old plants were analyzed for chlorophyll content, and overall rosette area was measured using ImageJ software. Additionally, percentage seed germination were calculated at various time points (3, 4, 5, and 6 days) after transferring to the growth room. After six days, root length were measured. Once the stress conditions are optimized, we plan to assess the oxidative stress levels of the plants using 3,3-diaminobenzidine (DAB) and Nitro blue tetrazolium (NBT) staining to detect hydrogen peroxide and superoxide, respectively. We will also investigate the levels of antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD), and peroxidase POD). Expression of the corresponding gene expression under stress conditions may also be monitored. To confirm the expression of Na^+ (HKT1 & NHX1) under NaCl stress, we will use CoroNaTM Green dye staining followed by confocal microscopy. Additionally, we will conduct computational analysis to characterize the LTP12 and determine its subcellular localization.

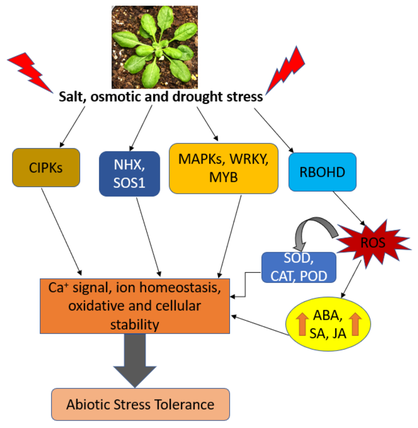


Figure 1. Schematic diagram of abiotic stress tolerance. CIPKs(CBL-Interacting Protein Kinases, NHX(Na^+/H^+ Exchanger), SOS1(Salt Overly Sensitive1), MAPKs (Mitogen Activated Protein Kinases), WRKY, MYB, RBOHD(Respiratory Burst Oxidase Homolog D, ROS(Reactive Oxygen Species), SOD(Superoxide Dismutase), CAT(Catalase), POD(Peroxidase), ABA(Abscisic Acid), SA(Salicylic Acid), JA(Jasmonic Acid)

PRELIMINARY RESULTS

To assess how well wild-type and mutant ltp12 Arabidopsis thaliana seeds can tolerate abiotic stress, they were germinated and transferred to growth chambers and tissue culture. Various analyses and assays were performed on the 3-week-old plants from both environments. The knockout ltp12 mutant exhibited promising preliminary results compared to the wildtype Col-0, suggesting the need for further research to determine its potential more conclusively for abiotic stress tolerance.

A. Percentage Seed Germination

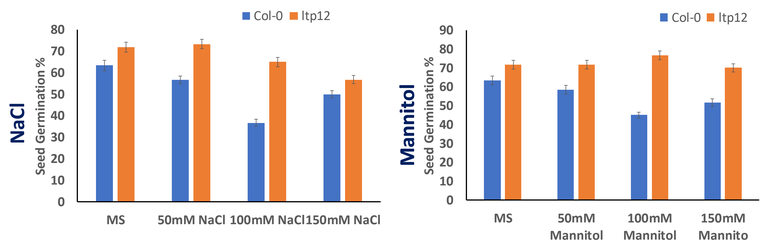


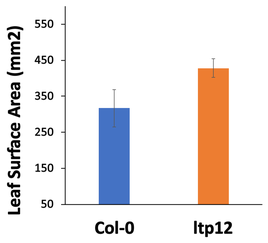
Figure 2: Percentage Seed germination. Overall, ltp12 mutant seeds showed higher seed germination compared to wildtype Col-0. The results presented here shows data from 2 independent experiments.

B. Morphological Phenotype of Mutant ltp12 and Wild-type Col-0

Figure 3: Morphological Phenotype of Mutant ltp12 and Wild-type Col-0. After 20 days of germination, rosette area of ltp12 were much larger and broader as compared to wildtype.

C. Leaf Surface Area of Col-0 and ltp12

Figure 4: Average leaf surface area Consistent with Fig. 3, the mutant ltp12 plants exhibited significantly larger leaf surface area compared to wildtype at 3 weeks of growth. Leaf surface area was measured on 5 leaves each from wildtype and mutant plants.



D. Root Growth in Salinity and Osmotic Stress

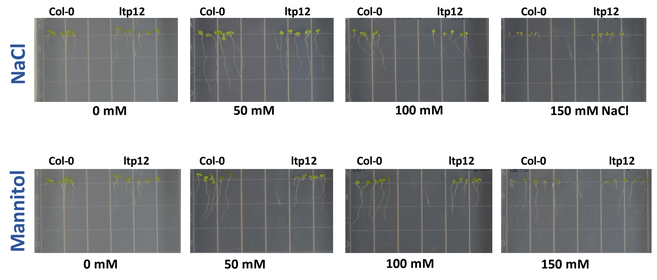


Figure 5: Root growth analysis. The wt Col-0 and the mutant ltp12 were grown in MS media with varying concentrations of NaCl and Mannitol. Pictures were taken 7 days stress.

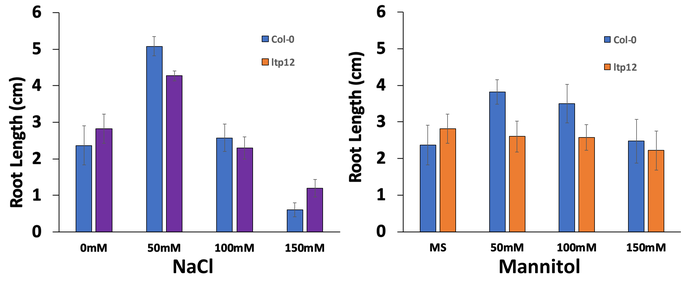


Figure 6: Root length analysis. After 7 days, both NaCl and Mannitol treatments at 150 mM resulted in significantly reduced root length, while the 50 mM concentration for both treatments exhibited the highest root length.

F. Chlorophyll Content Measurement

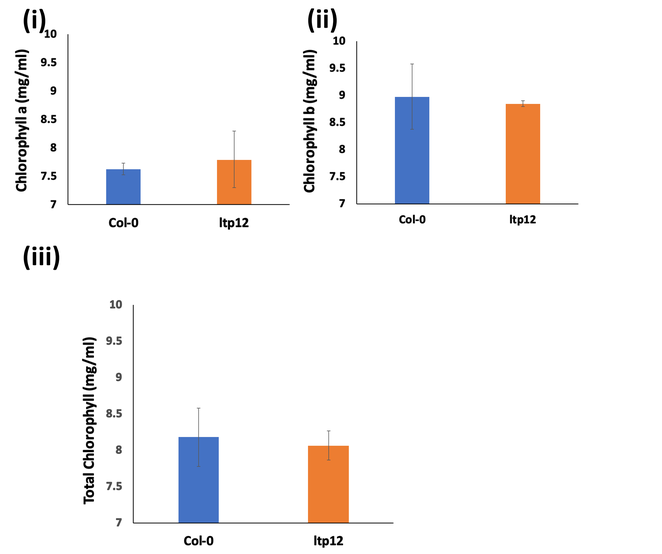


Figure 7: Chlorophyll content. Chlorophyll a (i), chlorophyl b (ii) and the total chlorophyll content (iii) was similar in wt Col-0 and the mutant ltp12 plants. The measurements were conducted using the leaves collected from 3 weeks old unstressed plants.

G. Average Water Loss in mutant vs wt *Arabidopsis* plants

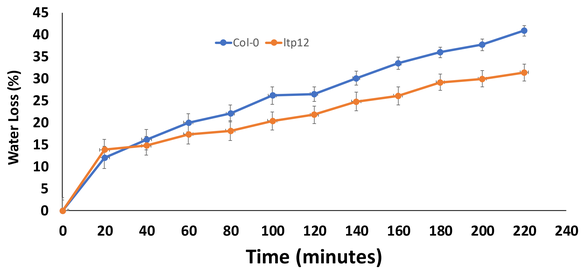


Figure 8: Water loss assay percentage. 3-week-old leaves of Col-0 and ltp12 at various time intervals were taken for water loss assay. Wildtype (Col-0) exhibited significant water loss, while ltp12 demonstrated promising results with less water loss, indicating tolerance capacity. 5 leaves each from wildtype and mutant were measured.

FUTURE DIRECTIONS

- Determine antioxidant enzymes (CAT, SOD and POD) levels
- Relative expression of antioxidant enzymes
- Tissue staining(DAB and NBT) to determine ROS levels
- Total soluble sugar estimation
- Sugar Reduction (Dinitro salicylic acid assay)
- Proline content analysis
- Confocal Microscopy (Na^+ homeostasis)
- Subcellular localization of LTP12

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