**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

**Functional studies of *Tectona grandis* genes involved in the formation of
wood and tolerance to environmental variation**

Perla Novais de Oliveira

Thesis presented to obtain the degree of Doctor in
Science. Area: Plant Physiology and Biochemistry

**Piracicaba
2020**

Perla Novais de Oliveira
Bachelor in Agronomy

Functional studies of *Tectona grandis* genes involved in the formation of wood and tolerance to environmental variation

Advisor:
Profa. Dra. **HELAINÉ CARRER**

Thesis presented to obtain the degree of Doctor in
Science. Area: Plant Physiology and Biochemistry

Piracicaba
2020

RESUMO

Estudos funcionais de genes de *Tectona grandis* envolvidos na formação da madeira e tolerância as variações ambientais

A madeira de teca (*Tectona grandis* L.) é uma das mais valorizadas do mundo e, apesar da expansão das plantações dessa espécie nos últimos 15 anos, pouco se sabe sobre sua dinâmica molecular tanto no que diz respeito à madeira, quanto sobre sua tolerância às variações ambientais. Novos procedimentos de engenharia genética, incluindo a caracterização funcional de genes, aparecem como alternativas para iniciar tais estudos. O transcriptoma que foi sequenciado e analisado no Laboratório de Genômica e Biologia Molecular da ESALQ / USP, mostrou a presença de genes (alguns deles como fatores de transcrição) relacionados à resposta ao estresse e ao crescimento da madeira. A avaliação da função desses genes transcritos e de suas interações moleculares, permite um melhor entendimento de como esses fenômenos ocorrem na espécie, potencializando o seu uso no melhoramento genético. Neste trabalho, estudos funcionais foram conduzidos para caracterizar os três genes selecionados, *TgMYB2*, *TgERF1* e *TgTPS1*. O *TgMYB2* foi isolado com sucesso de *T. grandis* e expresso constitutivamente em plantas de tabaco, para investigar seu papel na regulação da formação da parede secundária. Além disso, a caracterização de *TgMYB2* por expressão heteróloga, tem sido mostrada e fornecido evidências de seu importante papel na regulação das vias de biossíntese de celulose, hemicelulose e lignina. Além disso, foi caracterizado o novo gene *TgERF1* responsivo ao estresse abiótico, um fator de transcrição do tipo AP2 / ERF. A superexpressão de *TgERF1* no tabaco aumentou a tolerância ao estresse de sal e seca, alterando parâmetros fisio-bioquímicos e ativação de uma série de genes responsivos ao estresse em nível molecular. Dada a importância da trealose e da família TPS na resposta ao estresse, uma nova proteína TPS de classe II de *T. grandis* foi isolada e caracterizada. O fenótipo das plantas com superexpressão de *TgTPS1* foi semelhante ao WT sob condições de controle, mas as plantas transgênicas mostraram maior tolerância ao sal e ao estresse hídrico. Assim, esses resultados fornecem importantes insights para desvendar os mecanismos de formação da madeira e tolerância a estresses, podendo ser úteis para estudos futuros visando melhorar a produtividade e tolerância a variações ambientais utilizando abordagens biotecnológicas.

Palavras-chave: Teca, Crescimento secundário, Formação de madeira, Estresse salino, Estresse hídrico

ABSTRACT

Functional studies of *Tectona grandis* genes involved in the formation of wood and tolerance to environmental variation

Teak (*Tectona grandis* L.) is one of the most valued wood in the world and, despite the expansion of plantations of this species in the last 15 years, little is known about its molecular dynamics regarding both its wood, and their tolerance for environmental variations. New genetic engineering procedures, including functional characterization of genes, appear as alternatives to initiate such studies. The transcriptome that was sequenced and analyzed at the Laboratory of Genomics and Molecular Biology, ESALQ/USP, showed the presence of genes (some of them as transcription factors) related to response to stress and wood growth. The evaluation of function of these transcribed genes and their molecular interactions, allows a better understanding of how these phenomena occur in the species, enhancing the potential of using them in genetic improvement. In this work, functional studies were conducted to characterize the three selected genes, *TgMYB2*, *TgERF1* and *TgTPS1*. The *TgMYB2* was successfully isolated from *T. grandis* and expressed constitutively in tobacco plants in order to investigate its role in the regulation of secondary wall formation. Also, the characterization of *TgMYB2* by heterologous expression, has been shown and provided evidences for its important role in regulating cellulose, hemicellulose and lignin biosynthesis pathways. In addition, it was characterized the novel abiotics stress-responsive *TgERF1* gene, an AP2/ERF type transcription factor. Overexpression of *TgERF1* in tobacco enhanced tolerance to salt and drought stresses, altering physio-biochemical parameters, and activation of an array of stress-responsive genes at molecular level. Given the importance of trehalose and the TPS family in the response to stress, a novel class II TPS protein from *T. grandis* was isolated and characterized. The phenotype of *TgTPS1*-overexpressing tobacco plants was similar to the WT under control conditions, but the transgenic plants showed improved tolerance to salt and drought stress. Thus, these results provide relevant insights to unravel the mechanisms of wood formation and stress tolerance, potentially being useful for future studies aiming to improve productivity and tolerance for environmental variations using biotechnological approaches.

Keywords: Teak, Secondary growth, Wood formation, Salinity stress, Drought stress

INTRODUCTION

Tectona grandis L f, popularly known as teak, is a tree species with a big size when mature has a highly worldwide commercial value, is deciduous, diploid ($2n = 36$), belong to the Lamiaceae family, which includes species that have paniculate inflorescence and tubular corolla, usually bilabiated (Stone, 1970). It is native to the tropical and subtropical regions of Southeast Asia, mainly India, Myanmar and Thailand, Laos and Indonesia (Java Island) (Daquinta et al. 2001), regions where teak was started to be planted to reduce pressure on the natural forest. Subsequently, commercial cultivation began in new tropical areas, particularly in West Africa, Central America and South America, especially in Costa Rica and Brazil, where plantations are characterized by high density of individuals and with shorter rotations than those practiced in Southeast Asia (Figueiredo et al., 2005).

Teak wood is the main commercial attraction of this species. Thanks to a wide variety of end uses, such as carpentry, rustic furniture joinery, manufacturing of laminates and floors, shipbuilding lumber and raw materials for handicrafts; its wood is versatile and technologically necessary for various uses, thus becoming a high quality furniture and a standard wood in the final classification of other tropical woods (Oliveira et al., 2007). The interest of many countries to include teak in the timber industry and in reforestation programs is mainly due to the high demand for its wood, high durability, dimensional stability, high proportions of sapwood and resistance to the external environment (Galeano et al., 2015).

Currently, teak is grown in more than 36 countries with 5.7 million hectares planted worldwide (Santos et al., 2014). Brazil introduced this species at the beginning of the 20th century, in the municipality of Cáceres (Mato Grosso), and the states of Mato Grosso, Amazonas, Pará, Acre and Maranhão are currently the main producers (Kollert; Cherubini, 2012). Adequate climatic conditions for the full development of teak in Brazil provide higher growth rates than the plantations of most wood-producing countries, with its cutting cycle reduced from 80 years (in the countries of origin) down to 25 years (Floréz, 2012). In addition, teak has gained prominence in Brazil, being an alternative in the replacement of native species of great economic value, reducing the pressure of exploitation of native forest ecosystems, such as the Amazon Forest, as well as for having utility for agrosilvopastoral production systems. and recovery of degraded areas (Healey; Gara, 2003; Moretti et al., 2014). Teak has been slightly improved conventionally for several decades due to its long reproduction time (Giri et al., 2004). New genetic engineering procedures, including gene manipulation technologies, appear as alternatives to increase forest productivity

(Poupin; Arce-johnson, 2005), wood quality and other desirable properties, such as resistance to pests and pathogens, stress and herbicides.

The transcriptome that was sequenced and analyzed at the Laboratory of Genomics and Molecular Biology, ESALQ/USP, showed the presence of genes (some of them as transcription factors) related to response to stress and wood growth. A better understanding of how these phenomena occur in teak allows the use genes from the transcriptome for genetic transformation to increase the tolerance to different types of stress and promote the improvement of wood quality. In addition, be used as markers for assisted selection of plants in nursery. Therefore, it is essential to evaluate the function of these transcribed genes and their molecular interactions, enhancing the potential of using them in genetic improvement.

Therefore, functional studies were performed to characterize the selected genes for this work in relation to the molecular dynamics with respect to the wood formation and the tolerance to environmental variations. In the first chapter, a functional analysis of the *TgMYB2* transcription factor through the characterization of the 35S::TgMYB2 transgenic line in *N. tabacum* showed its role in regulating the biosynthesis pathways of cellulose, hemicellulose and lignin. In the second chapter, since that it was verified that *TgERF1* gene expression was rapidly induced by polyethylene glycol (PEG), NaCl and exogenous treatment ABA, ACC and JA, it suggested a potential role in tolerance to salt and drought teak. Thus, the coding sequence of full-length (CDS) of *TgERF1* transcription factor was isolated from teak leaves, cloned and transformed into tobacco plants to investigate the functional role of *TgERF1* in regulating responses to drought and salt stress. In the third chapter, given the importance of trehalose and TPS family in response to stress, *TgTPS1* gene was isolated successfully from teak and also constitutively expressed in tobacco plants. The characterization of the 35S::TgTPS1 transgenic lines were carried out by establishing a basis for the study of TPS functions and research on stress tolerance in teak.

REFERENCES

- DAQUINTA, M.; RAMOS, L.; CAPOTE, I.; LEZCANO, Y.; RODRÍGUEZ, R.; TRINA, D.; ESCALONA, M. 2001. Micropropagación de la teca (*Tectona grandis* L.F.). **Revista Florestal Centroamericana**, Comunicación técnica, n. 35, p. 25-28.
- FIGUEIREDO, E.O.; OLIVEIRA, L.C.; BARBOZA, L.K. 2005. Teca (*Tectona grandis* L.f.): principais perguntas do futuro empreendedor florestal. Rio Branco: **Embrapa Acre**, 87p. il. Color. (Embrapa Acre. Documentos, 97).
- FLORÉZ, J. B. (2012). **Caracterização tecnológica da madeira jovem de teca (*Tectona grandis* L. f.)**. Universidade Federal de Lavras, 85 p. 2012.
- GALEANO, E.; VASCONCELOS, T. S.; VIDAL, M.; MEJIA-GUERRA, M. K.; CARRER, H. Large-scale transcriptional profiling of lignified tissues in *Tectona grandis*. **BMC Plant Biology**, v. 15, 1, p.221, 2015.

- GIRI, C.C.; SHYAMKUMAR, B.; ANJANEYULU, C. Progress in tissue culture, genetic transformation and applications of biotechnology to trees: An overview. **Trees -Structure and Function**, v.18, n.2, p.115–135, 2004.
- HEALEY, S. P.; GARA, R. I. The effect of a teak (*Tectona grandis*) plantation on the establishment of native species in an abandoned pasture in Costa Rica. **Forest Ecology and Management, Amsterdam**, v. 176, p. 497 – 507, 2003.
- KOLLERT, W.; CHERUBINI, L. **Teak resources and market assessment** 2010. Rome: Food and Agriculture Organization 2012, (FAO Planted Forests and Trees Working Paper FP/47/E).
- MORETTI, M. S. Crescimento inicial de plantas de teca em monocultivo e Sistema Taungya com milho em Figueiropolis D'Oeste, estado de Mato Grosso. **Scientia Forestalis, Piracicaba**, v. 42, n. 102, p. 269 – 277, 2014.
- OLIVEIRA, L. C.; ANGELI, A.; STAPE, J. L. Teca é a nova opção na indústria mundial. **Revista da Madeira**, Brasília, v. 106, 2007.
- POUPIN, M.J. ARCE-JOHNSON, P., 2005. Transgenic trees for a new era. **In Vitro Cellular & Developmental Biology**, v. 41, n. 2, p.91–101.
- SANTOS, A. F. A.; ALMEIDA, B. C.; GAVA, F. H.; FAVARE, H. G.; FILHO, J. B.; COSTA, R. B.; BRONDANI, G. E. Clones production of *Tectona grandis*. **Advances in Forestry Science** v.1, p.75-82, 2014.
- STONE, B.C. 1970. **The Flora of Guam. A Manual for the Identification of the Vascular Plants of the Island. Micronesica**. 6 (1/2): 1-659.

1. OVEREXPRESSION OF THE MYB FAMILY GENE *TgMYB2* IMPACTS BIOMASS ACCUMULATION AND SECONDARY CELL WALL IN TOBACCO PLANTS

ABSTRACT

A Teak is the tropical tree with the best-known decorative wood in the world, for its extraordinary qualities of color, density and durability. Despite the commercial interest, little is known about the molecular process of wood formation in teak. Recent molecular and genetic studies have identified the existence of a hierarchical regulation of secondary wall formation, involving hormonal regulators, transcription factors and regulators of cell division. Among them, the MYB family of transcription factors were found to be the master switches of secondary cell wall biosynthesis. Extensive analysis of the transcriptome of the secondary xylem stem and branch of teak from 12 and 60 years old has been reported. Within the large number of transcription factors identified with high expression in the secondary xylem of young stems, TgMYB2, was selected to shown how it contributes to woody growth in teak. Thus, the full-length coding sequence (CDS) of TgMYB2 was isolated from teak plants, cloned and transformed into tobacco plants to investigate its involvement in the regulation of secondary wall formation. In addition, we report the functional characterization of TgMYB2 and provide evidences for its important role in regulating cellulose, hemicellulose and lignin biosynthesis pathways.

Keywords: *Tectona grandis*, MYB transcription factors, Wood formation, Functional genomics

1.1 INTRODUCTION

Wood is of great importance to humans as a raw material for the production of biodiesel, fibers, solid wood products and various natural compounds, including pharmaceutical products (Scarlat et al., 2015). Despite the commercial interest and the large number of scientific researches, little is known about the molecular process of wood formation in tropical trees (Abril et al., 2011). Wood is a set of tissues derived from the secondary growth of the tree stem. Wood formation begins with the enlargement and cell division of the vascular cambium ring in the stem of developing trees, with a later differentiation of the secondary xylem (wood) towards the interior of the vascular cambium, and secondary phloem (inner shell) to the outside. This process includes the expansion of xylem cells, the deposition of the secondary cell walls and programmed cell death (Chaffey, 2002; Dharmawardhana et al., 2010; Liu et al., 2014).

In all vascular plants, the secondary cell wall is composed of cellulose and hemicellulose microfibrils embedded in a matrix of the polymer lignin forming a complex crosslink (Pauly & Keegstra, 2010; Loque et al. 2015). Recent molecular and genetic studies have identified the existence of a hierarchical regulation of secondary wall formation, involving hormonal regulators,

transcription factors and regulators of cell division (Matsumoto et al., 2008; Fornales et al. 2011; Shen et al., 2012).

Transcription factors play important roles in the regulation in plants, by regulating the expression of key genes involved in plant growth and development (Singh et al., 2002), through binding to cis acting elements in the promoters of downstream target genes or interacting with other transcription factors (Chen & Zhu 2004; Cheng et al. 2013). Among them, the NAC and MYB family of transcription factors were found to be master switches of secondary cell wall biosynthesis. MYB transcription factors comprise one of the largest transcription factor families distributed in all eukaryotic organisms. MYB family is defined by an N-terminal DNA binding domain (DBD), which is responsible for the regulatory activity of the protein, and a non-conserved C-terminal modulator region (Bedon et al., 2007). According to the number of the binding domain repeats, MYBs can be classified as 1R-MYB, R2R3-MYB, 3R-MYB and 4R-MYB (Baldoni et al., 2015). The large size of the MYB family indicates their importance in the control of plants physiological and biochemical processes, including the regulation of secondary metabolism (Bedon et al., 2007).

These proteins have been studied extensively during the past years. For instance, the overexpression of *PtMYB4* (R2R3-MYB) from *Pinus taeda* (Patzlaff et al., 2003) and *EgMYB2* from *Eucalyptus grandis* (Goicoechea et al., 2005), in transgenic tobacco plants, altered the accumulation of gene transcripts from the phenylpropanoid pathway and increased cell wall thickness and lignin deposition, indicating that they are involved in transcriptional regulation of the lignin biosynthetic pathway. In contrast, *VvMYB5a* from *Vitis vinifera* (Deluc et al., 2006) altered structural genes that control the synthesis of phenylpropanoids, and the overexpression of the *AmMYB308* and *AmMYB330* genes suppressed the biosynthesis of lignin in tobacco, with at least 17% less lignin in vascular tissue (Jaffe et al., 2007). *PvMYB4* overexpression reduced lignin content, altered cell wall phenolic content, and reduced recalcitrance in transgenic grass (Shen et al., 2012).

Currently, several exotic tree species such as eucalyptus, pine and teak are being planted in Brazil, however teak (*Tectona grandis* Lf) has stood out as one of the most promising species for planting in the southeastern and central-western regions of the country (Abraflor, 2009). Teak is not a fast-growing species, but it can produce wood of excellent strength in relatively short rotations of 21 years (Bhat & Indira, 1997), depending on the sapwood-core percentages. It is the tropical tree with the best-known decorative wood in the world, for its extraordinary qualities of color, density and durability (Galeano et al., 2015). Recently, extensive analysis of the transcriptome of the secondary xylem stem and branch of teak from 12 and 60 years old has been reported (Galeano et al., 2015). Although a large number of transcription factors has been identified with

high expression in the secondary xylem of young stems, such as *TgMYB2* (Galeano et al., 2015), it is not yet known how they interact to influence woody growth in teak.

In this research, the full-length coding sequence (CDS) of *TgMYB2* was isolated from teak leaves and transformed into tobacco plants to investigate the role of *TgMYB2* in the regulation of secondary wall formation. Here, we report the functional characterization of *TgMYB2* and provide evidences for its important role in regulating cellulose, hemicellulose and lignin biosynthesis pathways.

1.2 CONCLUDING REMARKS AND FUTURE PROSPECTS

Here we isolated and characterized a novel 3R-MYB transcription factor, *TgMYB2*. It has been successfully isolated from *T. grandis* and expressed constitutively in tobacco plants. *TgMYB2* exhibits an exclusive expression in the nucleus, and does not present transmembrane motifs, which is in accordance with the role of its protein as a transcription factor. Overexpression of *TgMYB2* showed an active effect on plant growth and improved biomass production of transgenic plants compared to WT plants supported by morphological and physiological parameters, through a coordinated regulation of cellulose, hemicellulose and lignin biosynthesis. The results presented here demonstrated that overexpression of *TgMYB2* significantly modified secondary growth and increased our understanding of the role played by *TgMYB2* transcription factor in wood formation. Thus, our results provide interesting insights to unravel the mechanisms of wood formation, one of the most important biological processes in plants. These results may suggest the manipulation of MYB2 for increasing biomass production in plants with bioenergy potencial.

REFERENCES

- ABRAFLOR. **Florestas Plantadas no Brasil**. Brasília: [s.n.], 2009.
- ABRIL, N. et al. Proteomics research on forest trees, the most recalcitrant and orphan plant species. **Phytochemistry**, v. 72, n. 10, p. 1219–42, jul. 2011.
- ALI, N.; CHEN, H.; ZHANG, C.; KHAN, S. A.; GANDEKA, M.; XIE, D.; ZHUANG, W. Ectopic expression of *AbGLK1b* (GOLDEN2-like Transcription Factor) in arabidopsis coferes dual resistance to fungal and bacterial pathogens. **Genes**, v. 11, n.3, p. 343, 2020.
- ALLAN, A. C.; HELLENS, R. P.; LAING, W. A. MYB transcription factors that colour our fruit. **Trends in Plant Science**, v.13, p. 99–102, 2008.
- AMBAVARAM, M. M.; KRISHNAN, A.; TRIJATMIKO, K. R.; PEREIRA, A. Coordinated activation of cellulose and repression of lignin biosynthesis pathways in rice. **Plant Physiology**, v. 155, p. 916–931, 2011.
- APPENZELLER, L.; DOBLIN, M.; BARREIRO, R.; WANG, H.Y.; NIU, X. M.; KOLLIPARA, K.; CARRIGAN, L.; TOMES, D.; CHAPMAN, M.; DHUGGA, K. S. Cellulose synthesis in maize: isolation and expression analysis of the cellulose synthase (CesA) gene family. **Cellulose**, v.11, n.3–4, p. 287–299, 2004.

- BALDONI, E., GENGA, A. AND COMINELLI, E. Plant MYB transcription factors: their role in drought response mechanisms. **International Journal Molecular Science**, v. 16, p. 15811–15851, 2015.
- BEDON, F.; GRIMA-PETTENATI, J.; MACKAY, J. Conifer R2R3-MYB transcription factors: sequence analyses and gene expression in wood-forming tissues of white spruce (*Picea glauca*). **BMC plant biology**, v. 7, p. 17, 2007.
- BHAT, K. M.; INDIRA, E. P. Effect of faster growth on timber quality of teak. **KFRI Research Report** n.132, p. 8-9, 1997.
- BIEMELT, S.; TSCHIERSCH, H.; SONNEWALD, U. Impact of altered gibberellin metabolism on biomass accumulation, lignin biosynthesis, and photosynthesis in transgenic tobacco plants. **Plant Physiology**, v. 135, p. 254–265, 2004.
- BRANDON, A.G.; SCHELLER, H. V. Engineering of Bioenergy Crops: Dominant Genetic Approaches to Improve Polysaccharide Properties and Composition in Biomass. **Frontiers Plant Science**, v. 11, p. 282, 2020.
- BRASILEIRO, A. C. M.; CARNEIRO, V. T. C. **Manual de Transformação Genética de Plantas**. Brasília: Embrapa. 309p. 1998.
- CAPELLA-GUTIÉRREZ, S.; SILLA-MARTÍNEZ, J.M.; GABALDÓN, T. trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. **Bioinformatics**, v. 25, p.1972–1973, 2009.
- CHAFFEY, N. Why is there so little research into the cell biology of the secondary vascular system of trees? **New Phytologist**, v. 153, n. 2, p. 213–223, 2002.
- CHENG, M. C.; LIAO, P. M.; KUO, W. W.; LIN, T. P. The Arabidopsis ETHYLENE RESPONSE FACTOR1 Regulates abiotic stress-responsive gene expression by binding to different cis-acting elements in response to different stress signals. **Plant Physiology**, v. 162, p. 1566–1582, 2013.
- CHEN, W. J., ZHU, T. Networks of transcription factors with roles in environmental stress response. *Trends Plant Science*, v.9, p. 591–596, 2004.
- COOK, C. M.; DAUDI, A.; MILLAR, D. J.; BINDSCHEDLER, L. V.; KHAN, S.; BOLWELL, G. P.; DEVOTO, A. Transcriptional changes related to secondary wall formation in xylem of transgenic lines of tobacco altered for lignin or xylan content which show improved saccharification. **Phytochemistry**, v. 74, p. 79–89, 2012.
- DELANO, W. L. Pymol: An open-source molecular graphics tool. **CCP4 Newsletter On Protein Crystallography**, 40, 82-92, 2002.
- DELUC, L. et al. Characterization of a Grapevine R2R3-MYB Transcription Factor That Regulates the Phenylpropanoid Pathway. **Plant physiology**, v. 140, p. 499–511, 2006.
- DHARMAWARDHANA, P.; BRUNNER, A. M.; STRAUSS, S. H. Genome-wide transcriptome analysis of the transition from primary to secondary stem development in *Populus trichocarpa*. **BMC genomics**, v. 11, p. 150, 2010.
- DUBOS, C.; STRACKE, R.; GROTEWOLD, E.; WEISSHAAR, B.; MARTIN, C.; LEPINIEC, L. MYB transcription factors in Arabidopsis. **Trends Plant Science**, v. 15, p. 573–581, 2010.
- DU, H., LIANG, Z., ZHAO, S. The Evolutionary History of R2R3-MYB Proteins Across 50 Eukaryotes: New Insights Into Subfamily Classification and Expansion. **Scientific Reports**, v. 5, p. 11037, 2015.
- FORNALE, S.; CAPELLADES, M.; ENCINA, A. Altered lignin biosynthesis improves cellulosic bioethanol production in transgenic maize plants down-regulated for cinnamyl alcohol dehydrogenase. *Molecular Plant*, v. 5, p. 817–830, 2011.

- GABRIELSEN, O.S.; SENTENAC, A.; FROMAGEOT, P. Specific DNA binding by c-Myb: evidence for a double helix-turn-helix-related motif. **Science**, v. 253, n. 5024, p. 1140-1143, 1991.
- GALEANO, E.; VASCONCELOS, T.S.; VIDAL, M.; MEJIA-GUERRA, M.K.; CARRER, H. Large-scale transcriptional profiling of lignified tissues in *Tectona grandis*. **BMC Plant Biology**, v. 15, p. 221, 2015.
- GOICOECHEA, M. et al. EgMYB2, a new transcriptional activator from Eucalyptus xylem, regulates secondary cell wall formation and lignin biosynthesis. **The Plant journal : for cell and molecular biology**, v. 43, n. 4, p. 553–67, ago. 2005.
- GOLFIER P, VOLKERT C, HE F, RAUSCH T, WOLF S. Regulation of secondary cell wall biosynthesis by a NAC transcription factor from Miscanthus. **Plant Direct**, v. 1, n. 5, 2017.
- GUINDON, S.; DUFAYARD, J.-F.; LEFORT, V.; ANISIMOVA, M.; HORDIJK, W.; GASCUE, O. New algorithms and methods to estimate Maximum-Likelihood Phylogenies: Assessing the performance of PhyML 3.0. **System Biology**, v. 59, p.307–321, 2010.
- GUO, M., THOMAS, J., COLLINS, G. AND TIMMERMAN, M.C.P. Direct repression of KNOX loci by the ASYMMETRIC LEAVES1 complex of Arabidopsis. **Plant Cell**, v. 20, 48–58, 2008.
- HAGA, N.; KATO, K.; MURASE, M.; ARAKI, S.; KUBO, M.; DEMURA, T.; SUZUKI, K.; MULLER, I.; VOSS, U.; JURGUENS, G.; ITO, M. R1R2R3-Myb proteins positively regulate cytokinesis through activation of KNOLLE transcription in *Arabidopsis thaliana*. **Development**, v. 134, p. 1101–1110, 2007.
- HAGA, N. Mutations in MYB3R1 and MYB3R4 cause pleiotropic developmental defects and preferential down-regulation of multiple G2/M-specific genes in Arabidopsis. **Plant Physiology**, v. 157, p. 706–717, 2011.
- HOANG, D.T.; CHERNOMOR, O.; HAESELER, A.; MINH, B.Q.; VINH, L.S. UFBoot2: Improving the Ultrafast Bootstrap Approximation. **Molecular Biology Evolution**, v. 35, p. 518–522, 2017.
- HOLDEN, L.A. Comparison of methods of in vitro dry matter digestibility for ten feeds. **Journal of Dairy Science**, v.82, p.1791-1794, 1999.
- HUANG, Y. et al. Differential activation of anthocyanin biosynthesis in Arabidopsis and tobacco over-expressing an R2R3 MYB from Chinese bayberry. **Plant Cell, Tissue and Organ Culture (PCTOC)**, v. 113, n. 3, p. 491–499, 26 jan. 2013.
- ITO M. G2/M-phase-specific transcription during the plant cell cycle is mediated by c-Myb-like transcription factors. **Plant Cell** 13:1891–1905, 2001.
- ITO, M. Conservation and diversification of three-repeat Myb transcription factors in plants. **Journal Plant Res.** v.118, p. 61–69, 2005.
- JAFFE, F. W.; TATTERSALL, A.; GLOVER, B. J. A truncated MYB transcription factor from *Antirrhinum majus* regulates epidermal cell outgrowth. **Journal of Experimental Botany**, v. 58, n. 6, p. 1515–1524, 2007.
- JARADAT, M.R.; FEURTADO, J.A.; HUANG, D.; CUTLER, A. J. Multiple roles of the transcription factor AtMYB1/AtMYB44 in ABA signaling, stress responses, and leaf senescence. **BMC Plant Biology**, v. 13, p. 192, 2013.
- JIANG, C-K.; RAO, G-Y. Insights into the Diversification and Evolution of R2R3-MYB. Transcription Factors in Plants, 2020. DOI: <https://doi.org/10.1104/pp.19.01082>
- JIANG, C. GU, J., CHOPRA, S., GU, X. & PETERSON, T. Ordered origin of the typical two- and three-repeat Myb genes. **Gene**, v. 326, p.13–22, 2004.
- JOSHUA, T. M.; MORGAN, J. L. W.; ZIMMER, J. A molecular description of cellulose biosynthesis. **Annual Review Biochemistry**, v. 84, p. 895-921, 2015.

- KATİYAR, A.; SMITA, S.; LENKA, S.K.; RAJWANSHI, R.; CHINNUSAMY, V.; BANSAL, K.C. Genome-wide classification and expression analysis of MYB transcription factor families in rice and Arabidopsis. **BMC Genomics** v. 13, p.544, 2012.
- KATO, K. Preferential up-regulation of G2/M phase-specific genes by overexpression of the hyperactive form of NtmybA2 lacking its negative regulation domain in tobacco BY-2 cells. **Plant Physiol.** 149:1945–1957, 2009.
- KATO, K.; STANDLEY, D.M. MAFFT. Multiple Sequence Alignment Software Version 7: Improvements in performance and usability. **Molecular Biology Evolution**, v.30, p. 772–780, 2013.
- LAWSON, T.; LEFEBVRE, S.; BAKER, N. R.; MORISON, J. I. L.; RAINES, C. A. Reductions in mesophyll and guard cell photosynthesis impact on the control of stomatal responses to light and CO₂. **Journal of Experimental Botany**, v.59, n. 13, p. 3609–3619, 2008.
- LEE, C.; ZHONG, R.; YE, Z. H. Biochemical characterization of xylan xylosyltransferases involved in wood formation in poplar. **Plant Signal**, v. 7, p.332–337, 2012.
- LEGAY, S.; SIVADON, P.; BLERVACQ, A. S.; PAVY, N.; BAGHDADY, A.; TREMBLAY, L.; LEVASSEUR, C.; LADOUCÉ, N.; LAPIERRE, C.; SEGUINGRIMA PETTENATI, J. EgMYB1, an R2R3 MYB transcription factor from eucalyptus negatively regulates secondary cell wall formation in arabidopsis and poplar. **New Phytology**, v. 188, n. 3, p. 774–786, 2010.
- LIU, L.; FILKOV, V.; GROOVER, A. Modeling transcriptional networks regulating secondary growth and wood formation in forest trees. **Physiologia plantarum**, v. 151, n. 2, p. 156–63, jun. 2014.
- LIU, J.; OSBOURN, A.; MA, P. MYB transcription factors as regulators of phenylpropanoid metabolism in plants. **Molecular Plants**, v. 8, n. 5, p. 689–708, 2015.
- LIU, Y.; WEI, M.; HOU, C.; LU, T.; LIU, L.; WEI, H.; CHENG, Y.; WEI, Z. Functional characterization of *populus PsnSHN2* in coordinated regulation of secondary wall components in tobacco. **Scientific Reports**, v. 42, 2017.
- LIVAK, K. J.; SCHMITTGEN, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta Delta C(T)). **Method. Methods**, v. 25, n. 4, p. 402–408, 2001.
- LOQUE, D.; SCHELLER, H. V.; PAULY, M. Engineering of plant cell walls for enhanced biofuel production. **Currents Opinion Plant Biology**, v. 25, p. 151–201, 2015.
- LU, T.; LIU, L.; WEI, M.; LIU, Y.; QU, Z.; WEI, C. Y. H.; WEI, Z. The effect of poplar *PsnGS1.2* overexpression on growth secondary cell wall, and fibre characteristics in tobacco. **Frontiers in Plant Science**, v.9, n. 9, 2018.
- MA, D.; CONSTABEL, C. P. MYB repressors as regulators of phenylpropanoid metabolism in plants. **Trends Plant Science**, v. 24, n. 3, p. 275–289, 2019.
- MATSUMOTO-KITANO M, KUSUMOTO T, TARKOWSKI P. Cytokinins are central regulators of cambial activity. **Proc Natl Acad Sci USA**, v. 105, p. 20027–20031, 2008.
- MCMANARA, J. T.; MORGAN, J. L. W.; ZIMMER, J. A molecular description of cellulose biosynthesis. **Annual review of biochemistry**, v. 84, p. 895–921, 2015.
- NOVAES, E.; OSORIO, L.; DROST, D. R.; MILES, B. L.; BOAVENTURA-NOVAES, C. R.; BENEDICT, C.; DERVINIS, C.; YU, Q.; SYKES, R.; DAVIS, M. Quantitative genetic analysis of biomass and wood chemistry of *Populus* under different nitrogen levels. **New Phytology**, v.182, p. 878–890, 2009.
- OGATA K. Solution structure of a DNA-binding unit of Myb: a helix-turn-helix-related motif with conserved tryptophans forming a hydrophobic core. **Proc Natl Acad Sci U S A**, v.89, p. 6428–6432, 1992.

- OMER, S.; KUMAR, S.; KHAN, B. M. Over-expression of a subgroup 4 R2R3 type MYB transcription factor gene from *Leucaena leucocephala* reduces lignin content in transgenic tobacco. **Plant Cell Reports**, v. 32, p. 161–171, 2013.
- PAULY, M.; KEEGSTRA, K. Plant cell wall polymers as precursors for biofuels. **Currents Opinion Plant Biology**, v. 13, n. 3, p. 305–317, 2010.
- PATZLAFF, A. et al. Characterisation of a pine MYB that regulates lignification. **The Plant Journal**, v. 36, n. 6, p. 743–754, dez. 2003.
- RAES J, ROHDE A, CHRISTENSEN JH, VAN DE PEER Y, BOERJAN W. Genome-wide characterization of the lignification toolbox in Arabidopsis. *Plant Physiology*, v. 133, v.3, p.1051–1071, 2003.
- ROSINSKI, J.A.; ATCHLEY, W.R. Molecular evolution of the Myb family of transcription factors: evidence for polyphyletic origin. **Journal Molecular Evolution**, v. 46, p. 74–83, 1998.
- SCARLAT, N.; DALLEMAND, J. F.; FERRARIO, F. M.; NITA, V. The role of biomass and bioenergy in a future bioeconomy: policies and facts. **Environmental Development**, v. 15, p. 3–34, 2015.
- SINGH, K.; FOLEY, R. C.; ONATE-SANCHEZ, L. Transcription factors in plant defense and stress responses. **Current Opinion in Plant Biology**, v. 5, n. 5, p. 430–436, 2002.
- SHEN, H.; HE, X.; POOVAIAH, C. R.; WUDDINEH, W. A.; MA, J.; MANN, D. G.; WANG, H.; JACKSON, L.; TANG, Y.; NEAL, S. C. Functional characterization of the switchgrass (*Panicum virgatum*) R2R3-MYB transcription factor PvMYB4 for improvement of lignocellulosic feedstocks. **New Phytologist**, v. 193, n. 1, p. 121–136, 2012.
- SOMERVILLE, C. Cellulose synthesis in higher plants. **Annual Review Cell Development Biology**, v.22, p. 53–78, 2006.
- SULLIVAN, J.; JOYCE, P. Model selection in phylogenetics. **Annual Review. Ecology Evolutive System**, v. 36, 445–446, 2005.
- TIAN F, YANG DC, MENG YQ, JIN JP AND GAO G. Plant RegMap: charting functional regulatory maps in plants. **Nucleic Acids Research**, gkz1020, 2019.
- TIMOTHY L. BAILEY, MIKAEL BODÉN, FABIAN A. BUSKE, MARTIN FRITH, CHARLES E. GRANT, LUCA CLEMENTI, JINGYUAN REN, WILFRED W. LI, WILLIAM S. Noble, "MEME SUITE: tools for motif discovery and searching", **Nucleic Acids Research**, v. 37: p.202–p208, 2009.
- WANG, W.; YUAN, Y.; YANG, C.; GENG, S.; SUN, Q.; LONG, L.; CAI, C.; CHU, Z.; LIU, X.; WANG, G.; DU, X.; MIAO, C.; ZHANG, X.; CAI, Y. Characterization, Expression, and Functional Analysis of a Novel NAC Gene Associated with Resistance to Verticillium Wilt and Abiotic Stress in Cotton. **G3 (Bethesda)**, v.7, n. 6, p.3951–3961, 2016.
- WATERHOUSE, A.; BERTONI, M.; BIENERT, S.; STUDER, G.; TAURIELLO, G.; GUMIENNY, R.; HEER, F.T.; DE BEER, T.A.P.; REMPFER, C.; BORDOLI, L.; LEPORE, R.; SCHWEDE, T.; SWISS-MODEL: homology modelling of protein structures and complexes. **Nucleic Acids Res**, v. 46, n.1, p. 296–p.303, 2018.
- WU AM, HORNBLAD E, VOXEUR A, GERBER L, RIHOUEY C, LEROUGE P, MARCHANT A. Analysis of the Arabidopsis IRX9/IRX9-L and IRX14/IRX14-L pairs of glycosyltransferase genes reveals critical contributions to biosynthesis of the hemicellulose glucuronoxylan. **Plant Physiology**, v. 153, n.2, p.542–554, 2010.

- XIE, Z., LEE, E., LUCAS, J.R., MOROHASHI, K., LI, D., MURRAY, J.A.H., SACK, F.D.; GROTEWOLD, E.
Regulation of cell proliferation in the stomatal lineage by the Arabidopsis MYB FOUR LIPS via direct targeting of core cell cycle genes. **Plant Cell**, v.22, p. 2306–2321, 2010.
- ZHAO Q. Lignification: Flexibility, Biosynthesis and Regulation. **Trends Plant Science**, v. 21, n. 8, p. 713-721, 2016.

2. OVEREXPRESSION OF THE TEAK *TgERF1* GENE, AN AP2/ERF TYPE TRANSCRIPTION FACTOR, INCREASES TOLERANCE TO DROUGHT AND SALT IN TRANSGENIC TOBACCO

ABSTRACT

Tectona grandis Linn. f., popularly known as Teak (Lamiaceae family) is one of the most important wood sources due to its extraordinary qualities of color, density and durability, and is cultivated in tropical regions with a significant market around the world. Abiotic stresses, such as high salinity, drought, cold and alkalinity, are an increasingly common and worrying environmental phenomena because it causes a loss of production in both agriculture and forestry. Plants adapt to these stress conditions by activation or repression of specific genes, and synthesize a large number of stress proteins to maintain their cellular function. Transcription factors play important roles in the regulation of these responsive genes in plants. For example, AP2/ERF (APETALA2/ethylene response factor), was found to be involved in stress signal transduction. Search in the teak transcriptome database, we identified an AP2/ERF gene that contain a key AP2/ERF domain named TgERF1. We demonstrated that the expression of TgERF1 was rapidly induced by Polyethylene Glycol (PEG), NaCl, and exogenous ABA, ACC and JA treatment, suggesting a potential role in drought and salt tolerance in teak. A functional analyzes was performed. The full-length coding sequence (CDS) of TgERF1 was isolated from teak leaves and cloned in tobacco plants to investigate the role of TgERF1 in the regulation of responses to drought and salt stress. Also, we report the functional characterization of TgERF1 and provide evidences that TgERF1 is a promising candidate gene be used as selective marker on plant breeding in order to improve plant stress tolerance.

Keywords: AP2/ERF family, Drought stress, Salt stress

2.1 INTRODUCTION

Abiotic stresses, such as high salinity, drought, cold and alkalinity, are the most common factors that restrict plant growth, development and crop yields (Thirumalaikumar et al., 2017; Zhang et al., 2018). Plants adapt to these stress conditions by altering physiological, morphological and molecular levels (Rasheed et al., 2016). When subjected to environmental stress, they activate multiple defense mechanisms through activation or repression of specific genes, expression and synthesize a large number of stress proteins to maintain cellular function in stress response and plant defense (Demekamp & Smeekens, 2003; Fujita et al., 2006).

Transcription factors play important roles in the regulation of these responsive genes in plants (Fujita et al., 2006), through binding to cis acting elements in the promoters of downstream target genes or by interacting with other transcription factors (Chen & Zhu 2004; Cheng et al. 2013). For example, WRKY, NAC (NAM, ATAF1, 2, and CUC2), HLH (base helix-loop-helix transcription factors), MYB (myeloblastosis oncogene), bZIP (basic leucine zipper) and AP2/ERF

(APETALA2/ethylene response factor), were found to play important roles in stress signal transduction (Nakashima et al., 2012; Singh et al., 2002).

AP2/ERF transcription factors belong to one of the largest plant kingdom transcription factor families, and are characterized by the ~ 60 amino acid AP2/ERF domain (Nakano et al., 2006), through which it binds to various dehydration-responsive element/C-repeat (DRE/CRT) and the GCC box (Fujimoto et al., 2000). AP2/ERF TFs have been shown to play essential roles in multiple biological processes that regulate plant growth and development (Dietz et al., 2010), as well as responses to environmental stresses (Sharma et al., 2010; Nakano et al., 2006). Results have shown that the expression levels of these genes can be induced by environmental signals such as drought, salt, cold and other stresses, are often mediated by hormonal signaling (Karaba et al., 2007; Cheng et al., 2013; Upadhyay et al., 2013; Zhang et al., 2018; Li et al., 2019). AP2/ERF superfamily have been classified into groups based on their sequence similarities and numbers of AP2/ERF domains: AP2, CBF/DREB, ERF and RAV (Sakuma et al., 2002; Nakano et al., 2006).

The ethylene-responsive factor (ERF) proteins were first reported in tobacco (Ohme-Takagi & Shinshi, 1995), since then, a number of ERF proteins have been identified in a series of plant species, such as arabidopsis, tomato, rice, poplar, eucalyptus and apple (Tournier et al., 2003; Nakano et al., 2004; Nakano et al., 2006; Chen et al., 2012; Girardi et al., 2013; Cao et al., 2015; Mawlong et al., 2015). ERFs proteins contain a highly conserved DNA binding domain (ERF domain) that interact with the GCC-box; and play important roles in responses to biotic stress (Hao et al., 1998). However, ERF protein also binds to DRE/CRT, mainly involved in responses to drought, high salt and extreme temperature (Jung et al., 2006; Kagale & Rozwadowski, 2011). These proteins have been studied extensively during the past years. For instance, over-expression of *Jatropha curcas* *JcERF* gene in Arabidopsis increased salt tolerance (Tang et al., 2007). Over-expression of *TaERF3*, an AP2/ERF type transcription factor, significantly increased salt and drought tolerance of transgenic wheat (Rong et al., 2014). It has been reported that overexpression of the soybean *GmERF3* gene in tobacco can enhance salt and drought stress tolerance (Zhang et al., 2009).

Tectona grandis Linn. f., popularly known as Teak (Lamiaceae family) is one of the most important wood sources due to its extraordinary qualities of color, density and durability, and is cultivated in tropical regions with a significant market around the world (Galeano et al., 2015). Abiotic stress is an increasingly common and worrying phenomenon because it causes a loss of production in both agriculture and forestry. Although teak is a tropical tree, which require alternating rainy and dry seasons to produce high-quality wood, planting teak in areas of water lack

is of concern, given their relatively high transpiration rates and reduced diameter growth (Sinacore et al., 2019).

Previously, in the teak transcriptome database, we identified an AP2/ERF gene that contain a key AP2/ERF domain named TgERF1 (GenBank accession No. MH003850.1). We have found expression of TgERF1 was rapidly induced by Polyethylene Glycol (PEG), NaCl, and exogenous ABA, ACC and JA treatment, suggesting a potential role in drought and salt tolerance in teak. In this research, the full-length coding sequence (CDS) of *TgERF1* was isolated from teak leaves and cloned in tobacco plants to investigate the role of TgERF1 in the regulation of responses to drought and salt stress. In addition, we report the functional characterization of TgERF1 and provide evidences of its important role in drought and salt stress response in tobacco plants.

2.2 CONCLUDING REMARKS AND FUTURE PROSPECTS

The gene *TgERF1*, which encodes an AP2/ERF transcription factor has been successfully isolated from *T. grandis*, and the transgenic lines were subjected to multiple abiotic stress and phytohormone treatments (PEG, NaCl, ABA, ACC and MeJA). The expression of *TgERF1* was exclusive to the nucleus, what is in accordance to the fact that this gene does not present transmembrane motifs and with its role as transcription factor. Overexpression of *TgERF1* in tobacco enhanced tolerance to salt and drought stresses. The improved stress tolerance of transgenic plants compared to control plants was supported by morphological and physio-biochemical parameters, and activation of an array of stress-responsive genes at molecular level, leading to synthesis of protective compounds. The results presented here demonstrate that overexpression of *TgERF1* significantly altered drought tolerance in the transgenic plants, and increased our understanding of the role played by TgERF1 transcription factor in responses to abiotic stresses. All the evidences presented here indicates that *TgERF1* is a promising candidate gene be used as selective marker on plant breeding in order to improve plant stress tolerance.

REFERENCES

- ACOSTA-MOTOS, J.R.; DÍAZ-VIVANCOS, P.; ÁLVAREZ, S.; FERNÁNDEZ-GARCÍA, N.; SÁNCHEZ-BLANCO, M.J.; HERNÁNDEZ, J.A. Physiological and biochemical mechanisms of the ornamental *Eugenia myrtifolia* L. plants for coping with NaCl stress and recovery. **Planta**, 242, 829–846, 2015.
- ALBACETE, A.; GHANEM, M. E.; MARTÍNEZ-ANDÚJAR, C.; ACOSTA M.; SÁNCHEZ-BRAVO, J.; MARTÍNEZ, V.; LUTTS, S.; DODD, I. C.; PÉREZ-ALFOCEA, F. Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (*Solanum lycopersicum* L.) plants. **Journal of Experimental Botany**, v. 59, n.15, p. 4119-4131, 2008.

- ALI, N.; CHEN, H.; ZHANG, C.; KHAN, S. A.; GANDEKA, M.; XIE, D.; ZHUANG, W. Ectopic expression of *AbGLK1b* (GOLDEN2-like Transcription Factor) in arabidopsis confers dual resistance to fungal and bacterial pathogens. **Genes**, v. 11, n.3, p. 343, 2020.
- ASHRAF, M.; FOOLAD, M.R. Roles of glycine betaine and proline in improving plant abiotic stress resistance. **Environment Experimental**, v. 59, p. 206–216, 2007.
- BAILEY, T.L.; JOHNSON, J.; GRANT, C.E.; NOBLE, W.S. The MEME suite. **Nucleic Acids Res**, 43, p.39–p.49, 2015.
- BATES, L. S.; WALDREN, R. P.; TEARE, I. D. Rapid determination of free proline for water-stress studies. **Plant Soil**, v. 39, p. 205–207, 1973.
- BRASILEIRO, A. C. M.; CARNEIRO, V. T. C. **Manual de Transformação Genética de Plantas**. Brasília: Embrapa. 309p. 1998.
- CAO, P. B.; AZAR, S.; SANCLEMENTE, H.; MOUNET, F.; DUNAND, C.; MARQUE, G. Genome-wide analysis of the AP2/ERF family in *Eucalyptus grandis*: an intriguing over-representation of stress-responsive DREB1/CBF Genes. **Plos One**, v. 10, n.e0121041, 2015.
- CAPELLA-GUTIÉRREZ, S.; SILLA-MARTÍNEZ, J.M.; GABALDÓN, T. TRIMAL: A tool for automated alignment trimming in large-scale phylogenetic analyses. **Bioinformatics**, v. 25, p. 1972–1973, 2009.
- CHENG, C. Y.; KRISHNAKUMAR, V.; CHAN, A. P.; THIBAUD-NISSEN, F.; SCHOBEL, S.; TOWN, C. D. Araport11: a complete reannotation of the *Arabidopsis thaliana* reference genome. *Plant Journal*, v. 89, n. 4, p.789-804, 2017.
- CHENG, M. C.; LIAO, P. M.; KUO, W. W.; LIN, T. P. The *Arabidopsis* ETHYLENE RESPONSE FACTOR1 Regulates abiotic stress-responsive gene expression by binding to different cis-acting elements in response to different stress signals. **Plant Physiology**, v. 162, p. 1566–1582, 2013.
- CHEN, Y.; HAN, Y.; ZHANG, M.; ZHOU, S.; KONG, X.; WANG, W. Overexpression of the wheat expansin gene TaEXPA2 improved seed production and drought tolerance in transgenic tobacco plants. **PLOS ONE**, v. 13, 2016.
- CHENG, Z.; ZHANG, X.; ZHAO, K.; YAO, W.; LI, R.; ZHOU, B.; JIANG, T. Over-Expression of ERF38 Gene Enhances Salt and Osmotic Tolerance in Transgenic Poplar. **Frontiers Plant Science**, v. 10, p. 1375, 2019.
- CHEN, W. J., ZHU, T. Networks of transcription factors with roles in environmental stress response. *Trends Plant Science*, v.9, p. 591–596, 2004.
- CHEN, N.; YANG, Q.; SU, M. Cloning of Six ERF family transcription factor genes from peanut and analysis of their expression during abiotic stress. **Plant molecular biology reports**, v. 30, p. 1415–1425, v. 2012.
- CHI, Y.J.; YANG, Y.; ZHOU, Y.; ZHOU, J.; FAN, B. F.; YU, J. Q. Protein-protein interactions in the regulation of WRKY transcription factors. **Molecular Plant**, v. 6, p. 287–300, 2013.
- DAVIS, J. C.; PETROV, D. A. Preferential duplication of conserved proteins in eukaryotic genomes. **Plos Biology**, v. 2, 2004.
- DEMEKAMP, M.; SMEEKENS, S.C. Integration of wounding and osmotic stress signals determines the expression of the AtMYB102 transcription factor gene. **Plant Physiology**, v.132, p. 1415–1423, 2003.
- DIETZ, K. J.; VOGEL, M. O.; VIEHHAUSER, A. AP2/EREBP transcription factors are part of gene regulatory networks and integrate metabolic, hormonal and environmental signals in stress acclimation and retrograde signalling. **Protoplasma**, v. 245, p. 3–14, 2010.

- FUJIMOTO, S. Y.; OHTA, M.; USUI, A.; SHINSHI, H.; OHME-TAKAGI, M. Arabidopsis ethylene-responsive element binding factors act as transcriptional activators or repressors of GCC box mediated gene expression. **Plant Cell** v. 12, p. 393–404, 2000.
- FUJITA, M.; FUJITA, Y.; NOUTOSHI, Y.; TAKAHASHI, F.; NARUSAKA, Y.; YAMAGUCHI-SHINOZAKI, K. Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. **Currents Opinions Plant Biology**, v. 9, p. 436–42. 2006.
- GALEANO, E.; VASCONCELOS, T. S.; OLIVEIRA, P. N.; CARRER, H. Physiological and molecular responses to drought stress in teak (*Tectona grandis* L.f.). **Plos One**, v. 14, p. 1–26, 2019.
- GALEANO, E.; VASCONCELOS, T. S.; VIDAL, M.; MEJIA-GUERRA, M. K.; CARRER, H. Large-scale transcriptional profiling of lignified tissues in *Tectona grandis*. **BMC Plant Biology**, v. 15, 1, p.221, 2015.
- GIRARDI, C. L.; ROMBALDI, C. V.; DAL CERO, J.; NOBILE, P. M.; LAURENS, F.; BOUZAYEN, M.; QUECINI, V. Genome-wide analysis of the AP2/ERF superfamily in apple and transcriptional evidence of ERF involvement in scab pathogenesis. **Science Horticulture**, v. 151, p.112–121, 2013.
- HAO, D.; OHME-TAKAGI, M.; SARAI, A. Unique mode of GCC box recognition by the DNA-binding domain of ethylene-responsive element-binding factor (ERF domain) in plant. **Journal Biological Chemists**, v. 273, p. 26857–26861, 1998.
- HARE, P.D.; CRESS, W.A.; VAN STADEN, J. Dissecting the roles of osmolyte accumulation during stress. **Plant Cell Environ**, v. 21, p. 535–553, 1998.
- HASSAN, A. L.; PACURAR, A.; LÓPEZ-GRESA, M. P.; DONAT-TORRES, M. P.; LLINARES, J. V.; BOSCAIU, M. Effects of salt stress on three ecologically distinct plantago species. **Plos one**, v. 11, n. 8, 2016.
- HOANG, D.T.; CHERNOMOR, O.; HAESELER, A.; MINH, B.Q.; VINH, L.S. UFBoot2: Improving the Ultrafast Bootstrap Approximation. **Molecular Biology Evolutive**, v. 35, p. 518–522, 2017.
- HUANG, X. S.; LIU, J-H.; CHEN, X. J. Overexpression of PtrABF gene, a bZIP transcription factor isolated from *Poncirus trifoliata*, enhances dehydration and drought tolerance in tobacco via scavenging ROS and modulating expression of stress-responsive genes. **BMC Plant Biology**, v.10, p. 230, 2010.
- HUO, Y.; WANG, M.; WEI, Y.; XIA, Z. Overexpression of the Maize psbA Gene Enhances Drought Tolerance Through Regulating Antioxidant System, Photosynthetic Capability, and Stress Defense Gene Expression in Tobacco. **Frontiers in Plant Science**, v. 6, p.1223, 2015.
- HUNDERTMARK, M.; KINCHA, D. K. LEA (Late Embryogenesis Abundant) proteins and their encoding genes in *Arabidopsis thaliana*. **BMC Genomics**, v. 9, p. 118, 2008.
- IJAZ, R.; EJAZ, J.; GAO, S.; LIU, T.; IMTIAZ, M.; YE, Z. WANG, T. Overexpression of annexin gene AnnSp2, enhances drought and salt tolerance through modulation of ABA synthesis and scavenging ROS in tomato. **Scientific Reports**, v. 7, p. 12087, 2017.
- JIN, Z. L., TIAN, T., NAEEM, M. S., JILANI, G., ZHANG, F., AND ZHOU, W. J. Chlorophyll fluorescence responses to application of new herbicide ZJ0273 in winter oilseed rape species. **International Journal Agricola Biology**, v. 13, p. 43–50, 2011.
- JUNG, J.; WON, S. Y.; SUH, S. C.; KIM, H.; WING, R.; JEONG, Y.; HWANG, I.; KIM, M. The barley ERF-type transcription factor HvRAF confers enhanced pathogen resistance and salt tolerance in *Arabidopsis*. **Planta**, v.225, p. 575–588, 2006.
- KAGALE, S.; ROZWADOWSKI, K. EAR motif-mediated transcriptional repression in plants: an underlying mechanism for epigenetic regulation of gene expression. **Epigenetics**, v. 6, p. 141–146, 2011.

- KARABA, A.; DIXIT, S.; GRECO, R.; AHARONI, A.; KURNIAWAN, R.; TRIJATMIKO, K. R.; MARSCH-MARTINEZ, N.; KRISHNAN, A.; KARABA, N. N.; UDAYAKUMAR, M.; PEREIR, A. Improvement of water use efficiency in rice by expression of HARDY, an Arabidopsis drought and salt tolerance gene. **Proc. Natl Acad Sci**, v. 104, p. 15270-15275, 2007.
- KRAUSE, G. H.; WEIS, E. Chlorophyll fluorescence and photosynthesis. **Advectcity Enzymology Relat Areas Molecular Biology**, v. 42, p. 313–349, 1991.
- KOVACS, D.; KALMAR, E.; TOROK, Z.; TOMPA, P. Chaperone activity of ERD10 and ERD14, two disordered stress-related plant proteins. **Plant Physiology**, v. 147, 381–390, 2008.
- LI, Y.; ZHANG, H.; ZHANG, Q.; LIU, Q.; ZHAI, H.; ZHAO, N.; HE, S. An AP2/ERF gene, *IbRAP2-12*, from sweetpotato is involved in salt and drought tolerance in transgenic *Arabidopsis*. **Plant Science**, v. 281, 19–30, 2019.
- LIU, W.; ZHAO, B.G.; CHAO, Q.; WANG, B.; ZHANG, Q.; ZHANG, C.; LI, S.; JIN, F.; YANG, D.; LI, X. The Maize AP2/EREBP Transcription Factor ZmEREB160 Enhances Drought Tolerance in Arabidopsis. **Tropical Plant Biology**, v. 13, p. 251-261, 2020.
- LIU, C.; ZHANG, T. Expansion and stress responses of the AP2/EREBP superfamily in cotton. **BMC Genomics**, v. 18, p. 118, 2017.
- LIVAK, K. J.; SCHMITTGEN, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta Delta C(T)). **Method. Methods**, v. 25, n. 4, p. 402–408, 2001.
- MAWLONG, I.; ALI, K.; SRIVINASAN, R. Functional validation of a drought-responsive AP2/ERF family transcription factor-encoding gene from rice in Arabidopsis. **Molecular Breeding**, v.35, n. 163, 2015.
- MISHRA N. A. Chlorophyll Fluorescence: A Practical Approach to Study Ecophysiology of Green Plants. In: **Advances in plant ecophysiology techniques**, p 77–99, 2018.
- MIZOI, J.; SHINOZAKI, K.; YAMAGUCHI-SHINOZAKI, K. AP2/ERF family transcription factors in plant abiotic stress responses. **Gene Regulatory Mechanisms**, v. 1819, n. 2, p. 86-96, 2012.
- MÜLLER, P.; LI, X. P.; NIYOGI, K. K. Non-photochemical quenching. A response to excess light energy. **Plant Physiology**, v. 125, p.1558–1566, 2001.
- MAXWELL, K.; JOHNSON, G.N. Chlorophyll fluorescence: A practical guide. **Journal Experimental Botany**, v. 51, p. 659–668, 2000.
- NAKANO, T.; SUZUKI, K.; FUJIMURA, T.; SHINSHI, H. Genome-wide analysis of the ERF gene family in Arabidopsis and rice. **Plant Physiology**, v.140, p.411–432, 2006.
- NAKANO, T.; TSUJIMOTO, Y.; NATTO, Y.; SUZUKI, K.; FUJIMURA, T.; SHINSHI, H. Genome-wide analysis of the Arabidopsis and rice ERF gene families. **Plant Cell Physiology**, v. 45, p. 204–p.204, 2004.
- NAKASHIMA, K.; TAKASAKI, H.; MIZOI, J.; SHINOZAKI, K.; YAMAGUCHI-SHINOZAKI, K. NAC transcription factors in plant abiotic stress responses. **Biochim. Biophys.Acta**, v. 1819, p. 97–103, 2012.
- OHME-TAKAGI, M.; SHINSHI, H. Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element. **Plant Cell**, v. 7, p. 173–182, 1995.
- OKAMURO, J. K.; CASTER, B.; VILLARROEL, R.; VAN-MONTAGU, M.; JOFUKU, K. D. The AP2 domain of APETALA2 defines a large new family of DNA binding proteins in Arabidopsis. **Proceedings of the National Academy of Sciences**, v. 94, p. 7076–708, 1997.

- Ouyang, S.; Zhu, W.; Hamilton, J.; Lin, H.; Campbell, M.; Childs, K.; Thibaud-Nissen, F.; Malek, R. L.; Lee, Y.; Zheng, L.; Orvis, J.; Haas, B.; Wortman, J.; Buell, C. R. The TIGR Rice Genome Annotation Resource: improvements and new features. **Nucleic acids research**, v. 35, n. D883-7, 2007.
- PARK, J. M.; PARK, C. J.; LEE, S. B.; HAM, B. K.; SHIN, R.; PAEK, K. H. Overexpression of the tobacco Tsi1 gene encoding an EREBP/AP2-type transcription factor enhances resistance against pathogen attack and osmotic stress in tobacco. **Plant Cell**, v.5, p. 1035–1046, 2001.
- PEGUEROLES, C., LAURIE, S., AND ALBÀ, M. M. Accelerated evolution after gene duplication: a time-dependent process affecting just one copy. **Molecular Biology Evolution**, v. 30, p. 1830–1842, 2013.
- PIECZYNSKI, M.; MARCZEWSKI, W.; HENNIG, J. Down-regulation of CBP80 gene expression as strategy to engineer a drought-tolerant potato. **Plant Biotechnol Journal**, v. 11, p. 459–469, 2013.
- RAMBOD, A.; NOOR, A. S.; MAHMOOD, M.; ZETTY, N. B. Y.; NARGES, A.; MAHBOD, S.; ALIREZA, V.; NAHID, K. P. A.; MOHAMED, M. Role of ethylene and the APETALA 2/ethylene response factor superfamily in rice under various abiotic and biotic stress conditions. **Environmental and Experimental Botany**, 2017.
- SÁNCHEZ, R.; SERRA, F.; TÁRRAGA, J.; MEDINA, I.; CARBONELL, J.; PULIDO, L.; MARÍA, A.; CAPELLA-GUTIÉRREZ, S.; HUERTA-CEPAS, J.; GABALDÓN, T.; DOPAZO, J.; DOPAZO, H. Pylemon 2.0: suite of web-tools for molecular evolution, phylogenetics, phylogenomics and hypotheses testing. **Nucleic Acids Research**, v. 39, p. 470-474, 2011.
- SINACORE, K.; BRETON, C.; ASBJORNSEN, HEIDI.; HERNANDEZ-SANTANA, VIRGINIA.; HALL, J. S. Drought effects on *Tectona grandis* water regulation are mediated by thinning, but the effects of thinning are temporary. **Frontiers in Forests and Global Change**, v. 2, p. 82, 2019.
- SINGH, K.B.; FOLEY, R.C.; ONATE-SÁNCHEZ, L. Transcription factors in plant defense and stress responses. **Currents Opinion Plant Biology**, v. 5, p. 430–436, 2002.
- REIS, R. R.; CUNHA, B. A. D. B.; MARTINS, P. K.; MARTINS, M.; ALEKCEVETCH, J. C.; CHALFUN-JÚNIOR, A.; ANDRADE, A. C.; RIBEIRO, A. P.; QIND, F.; MIZOI, J.; YAMAGUCHI-SHINOZAKI, J.; NAKASHIMA, K.; CARVALHO, J. F. C.; SOUSA, C. A. F.; NEPOMUCENO, A. L.; KOBAYASHI, A. K.; H. B. C. Induced over-expression of AtDREB2A CA improves drought tolerance in sugarcane. **Plant Science**, v. 221, n. 222, p. 59–68, 2014.
- RASHEED, S.; BASHIR, K.; MATSUI, A.; TANAKA, M.; SEKI, M. Transcriptomic analysis of soil-grown *Arabidopsis thaliana* roots and shoots in response to a drought stress. *Frontiers Plant Science*, v. 7, p. 180, 2016.
- RIECHMANN, J. L.; HEARD, J.; MARTIN, G.; REUBER, L.; JIANG, C. Z.; KEDDIE, J. *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. **Science**, v. 290, p. 2105–2110, 2000.
- RONG, W.; QI, L.; WANG, A.; YE, X.; DU, L.; LIANG, H.; XIN, Z.; ZHANG, Z. The ERF transcription factor TaERF 3 promotes tolerance to salt and drought stresses in wheat. **Plant Biotechnology**, v. 12, p. 468–479, 2014.
- SAKUMA, Y.; LIU, Q.; DUBOUZET, J.G.; ABE, H.; SHINOZAKI, K.; YAMAGUCHI-SHINOZAKI, K. DNA-binding specificity of the ERF/AP2 domain of *Arabidopsis* DREBs:transcription factors involved in dehydration- and cold-inducible gene expression. **Biochem. Biophys. Res. Commun**, v. 290, p. 998–1009, 2002.

- SHARMA, V.; GOEL, P.; KUMAR, S. Na apple transcription factor, MdDREB76, confers salt and drought tolerance in transgenic tobacco by activating the expression of stress-responsive genes. **Plant Cell Reports**, v. 38, p. 221–241, 2019.
- SHARMA, M.K.; KUMAR, R.; SOLANKE, A.U.; SHARMA, R.; TYAGI, A.K.; SHARMA, A.K. Identification, phylogeny, and transcript profiling of ERF family genes during development and abiotic stress treatments in tomato. **Molecular Genetics Genomic**, v. 284, p. 455–475, 2010.
- SHU, Y.; LIU, Y.; ZHANG, J.; SONG, L.; GUO, C. Genome-Wide Analysis of the AP2/ERF Superfamily Genes and their Responses to Abiotic Stress in *Medicago truncatula*. **Frontiers Plant Science**, v. 6, p. 1247, 2016.
- SINGH, K.B.; FOLEY, R.C.; ONATE-SÁNCHEZ, L. Transcription factors in plant defense and stress responses. **Currents Opinion Plant Biology**, v. 5, p. 430–436, 2002.
- SIQUEIRA, M.; GOMES, L. Functional Diversity of Early Responsive to Dehydration (ERD) Genes in Soybean. **A Comprehensive Survey of International Soybean Research - Genetics, Physiology, Agronomy and Nitrogen Relationships**. <https://doi.org/10.5772/50993>, 2013.
- TANG, W.; CHARLES, T. M.; NEWTON, R. J. Overexpression of the pepper transcription factor CaPF1 in transgenic Virginia pine (*Pinus Virginiana* Mill.) confers multiple stress tolerance and enhances organ growth. **Plant Molecular Biology**, v. 59, p. 603–617, 2005.
- TANG, M.; SUN, J.; LIU, Y.; CHEN, F.; SHEN, S. Isolation and functional characterization of the JcERF gene, a putative AP2/EREBP domain-containing transcription factor, in the woody oil plant *Jatropha curcas*. **Plant Molecular Biology**, v. 63, p. 419–428, 2007.
- THIRUMALAIKUMAR, V.P.; DEVKAR, V.; MEHTEROV, N.; ALI, S.; OZGUR, R.; TURKAN, I. NAC transcription factor JUNGBRUNNEN1 enhances drought tolerance in tomato. **Plant Biotechnology**, v. 16, p. 354–366, 2017.
- TIAN F, YANG DC, MENG YQ, JIN JP AND GAO G. PlantRegMap: charting functional regulatory maps in plants. **Nucleic Acids Research**, gkz1020, 2019.
- TOURNIER, B.; SANCHEZ-BALLESTA, M. T.; JONES, B.; PESQUET, E.; RAGAD, F.; LATCHÉ, A.; PECH, J.C; BOUZAYEN, M. New members of the tomato ERF family show specific expression pattern and diverse DNA-binding capacity to the GCC box element. **FEBS Letters**, v. 550, p. 149–154, 2003.
- TRISHLA V. S.; KIRTI, P. B. Structure-function relationship of *Gossypium 1 hirsutum* NAC transcription factor, GhNAC4 with regard to ABA and abiotic stress responses.
- UPADHYAY, R. K.; SONI, D. K.; SINGH, R.; DWIVEDI, U. N.; PATHRE, U. V.; NATH, P.; SANE, A. P. SIERF36, an EAR-motif-containing ERF gene from tomato, alters stomatal density and modulates photosynthesis and growth. **Journal of Experimental Botany**, v. 64, n. 11, p. 3237–3247, 2013.
- WANG L.; MA, H.; LIN, J. Angiosperm-Wide and Family-Level Analyses of AP2/ERF Genes Reveal Differential Retention and Sequence Divergence After Whole-Genome Duplication. **Frontiers Plant Science**, v. 10, p. 196, 2019.
- WANG, W. B.; KIM, Y. H.; LEE, H. S.; KIM, K. Y.; DENG, X. P.; KWAK, S. S. Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. **Plant Physiol Biochem**, v. 47, p. 570–577, 2009.
- WANG, W.; YUAN, Y.; YANG, C.; GENG, S.; SUN, Q.; LONG, L.; CAI, C.; CHU, Z.; LIU, X.; WANG, G.; DU, X.; MIAO, C.; ZHANG, X.; CAI, Y. Characterization, Expression, and Functional Analysis of a Novel NAC

- Gene Associated with Resistance to Verticillium Wilt and Abiotic Stress in Cotton. **G3 (Bethesda)**, v.7, n. 6, p.3951-3961, 2016.
- WU, L.; CHEN, X.; REN, H.; ZHANG, Z.; ZHANG, H.; WANG, J.; WANG, X-C; HUANG, R. ERF protein JERF1 that transcriptionally modulates the expression of abscisic acid biosynthesis-related gene enhances the tolerance under salinity and cold in tobacco. **Planta**, v. 226, p. 815–825, 2007.
- WU, Y.; JIN, X.; LIAO, W.; HU, L.; DAWUDA, M. M.; ZHAO, X.; TANG, Z.; GONG, T.; YU, J. 5-Aminolevulinic Acid (ALA) Alleviated Salinity Stress in Cucumber Seedlings by Enhancing Chlorophyll Synthesis Pathway. **Frontiers in Plant Science**, v. 9, n. 635, 2018.
- XIA Z.; SU, X.; LIU, J.; WANG, M. The RING-H2 finger gene 1 (RHF1) encodes an E3 ubiquitin ligase and participates in drought stress response in *Nicotiana tabacum*. **Genetica**, v. 141, p. 11–21, 2013.
- XIE, Z.; NOLAN, T. M.; JIANG, H.; YIN, Y. AP2/ERF Transcription Factor Regulatory Networks in Hormone and Abiotic Stress Responses in *Arabidopsis*. **Frontiers Plant Science**, v.10, p. 228, 2019.
- XU, Z.; XIA, L.; CHEN, M.; CHENG, X.; ZHANG, R.; LI, L.; ZHAO, Y.; LU, Y.; NI, Z.; LIU, L.; QIU, Z.; MA, Y. Isolation and molecular characterization of the *Triticum aestivum* L. ethylene-responsive factor 1 (TaERF1) that increases multiple stress tolerance. **Plant Molecular Biology**, v. 65, p. 719–732, 2007.
- YAO, W.J.; WANG, L.; ZHOU, B.R.; WANG, S.J.; LI, R.H.; JIANG, T.B. Over-expression of poplar transcription factor ERF76 gene confers salt tolerance in transgenic tobacco. **Journal Plant Physiology**, v. 198, p. 23–31, 2016.
- YANG, Y.; DONG, C.; LI, X.; DU, J.; QIAN, M.; SUN, X.; YANG, Y. A novel AP2/ERF transcription factor from *Stipa purpurea* leads to enhanced drought tolerance in *Arabidopsis thaliana*. **Plant Cell Rep.** **2016**, 35, 2227–2239.
- YI, S. Y.; KIM, J. H.; JOUNG, Y. H.; LEE, S.; KIM, W. T.; YU, S. H. The pepper transcription factor CaPF1 confers pathogen and freezing tolerance in *Arabidopsis*. **Plant Physiology**, v. 136, p; 2862-2874, 2004.
- ZHANG, B.; SU, L.; HU, B.; LI, L. Expression of AhDREB1, an AP2/ERF Transcription Factor Gene from Peanut, Is Affected by Histone Acetylation and Increases Absciscic Acid Sensitivity and Tolerance to Osmotic Stress in *Arabidopsis*. **International Journal Molecular Science**, v. 19, n.5, p. 1441, 2018.
- ZHANG, G.; CHEN, M.; LI, L.; XU, Z.; CHEN, X.; GUO, J.; MA, Y. Overexpression of the soybean GmERF3 gene, an AP2/ERF type transcription factor for increased tolerances to salt, drought, and diseases in transgenic tobacco. **Journal of Experimental Botany**, v. 60, n. 13, p. 3781–3796, 2009.
- ZHAO, D.; HAMILTON, J. P.; BHAT, W. W.; JOHNSON, S. R.; GODDEN, G. T.; KINSER, T. J.; BOACHON, B.; DUDAREVA, N.; SOLTIS, D. E.; SOLTIS, P. E.; HAMBERG, B.; BUELL, C. R. A chromosomal-scale genome assembly of *Tectona grandis* reveals the importance of tandem gene duplication and enables discovery of genes in natural product biosynthetic pathways. **Giga Science**, v. 8., n. 3, 2019.
- ZHU, J.K. Salt and drought stress signal transduction in plants. **Annual Review Plant Biology**, v. 53, p. 247–273, 2002.

3. FUNCTIONAL CHARACTERIZATION OF TREHALOSE-6-PHOSPHATE SYNTHASE FAMILY GENE *TgTPS1* IN RESPONSE TO ABIOTIC STRESS IN TOBACCO PLANTS

ABSTRACT

Plants are continuously exposed to a great variety of abiotic stresses, such as drought, extreme temperature, high salinity and alkalinity, which lead to a restriction on plant growth and result in massive crop yields losses. Plant adaptation to these conditions involve the neutralization of the negative effects caused by these stresses, through activation or repression of specific genes expression, and synthesis of a large number of non-toxic organic compounds of low-molecular weight that are involved in cellular osmoregulation, one of them is the sugar trehalose. Among the important functions of trehalose, it mainly operates to protect the cellular proteins and membranes, as well as stabilizing the dehydrated enzymes. Previous research has shown that after the application of moderate and severe water stress on teak seedlings, the expression of *TgTPS1* (Teak Trehalose Phosphate Synthase) gene was always higher than the expression of plants submitted to control conditions. These results led us to further investigate the role of *TgTPS1* in plants under abiotic stress. In this research, the full-length coding sequence (CDS) of *TgTPS1* was isolated from teak leaves and cloned in tobacco plants to investigate its role in the regulation of responses to drought and salt stress. *TgTPS1* has been successfully isolated from teak and expressed constitutively in tobacco plants. The phenotype of *TgTPS1*-overexpressing plants and WT were similar under control conditions, but under stress conditions transgenic plants showed improved tolerance to high level of salt and drought. The improved stress tolerance of transgenic plants was supported by morphological and physio-biochemical parameters, and activation of an array of stress-responsive genes at molecular level. Thus, the results presented here contribute to establish a foundation for the study of TPS functions and its role on teak stress tolerance.

Keywords: Teak, Trehalose, Drought Stress, Salt Stress

3.1 INTRODUCTION

Plants are continuously exposed to a great variety of abiotic stresses, such as drought, extreme temperature, high salinity and alkalinity, which lead to a restriction on plant growth and result in massive crop yields losses (Fahad et al., 2017; Thirumalaikumar et al., 2017; Zhang et al. 2018). Plants adaptation to these conditions involve the neutralization of the negative effects caused by these stresses (Rasheed et al., 2016). When subjected to environmental stress, plants can activate multiple defense mechanisms through activation or repression of specific genes expression, and synthesis of a large number of non-toxic organic compounds of low-molecular weight that are involved in cellular osmoregulation (Fujita et al., 2006; Nadel et al., 2019).

Trehalose (α -D-glucopyranosyl- α -D-glucopyranoside), a non-reducing and soluble disaccharide, serves as a carbon source, signal metabolite and protection agent against stress (Wiemken, 1990; Strom & Kaasen 1993). These important functions of trehalose mainly operate by protecting the cellular proteins and membranes, as well as stabilizing the dehydrated enzymes (Kosar et al., 2019). In nature, trehalose is synthesized by diverse organisms as, bacteria, fungi,

algae, invertebrates and plants (Elbein, 1974). There are at least three different pathways for biological synthesis of trehalose. The most widespread in all the prokaryotic and eukaryotic organisms and the only found in plants (Paul et al., 2008) is the trehalose OtsA–OtsB pathway. Trehalose biosynthesis is a two-step enzymatic process, where trehalose-6-phosphate synthase (TPS) synthesizes trehalose-6-phosphate (T6P) from UDP-glucose (UDP-Glc) and glucose 6-phosphate (Glc-6-P), followed by dephosphorylation to trehalose by trehalose-6-phosphate phosphatase (TPP) (Paul 2007; Paul et al. 2008). Thus, TPS synthase is the key enzyme for trehalose biosynthesis as it regulates the levels of T6P, thereby affecting growth, developmental and metabolic processes in plants (Lunn et al., 2015).

TPS members in plants were classified into two classes (Leyman et al., 2001; Avonce et al., 2006). Class I includes TPS1-4 genes, homologous to the yeast *ScTPS1*, while class II includes TPS5-11 genes, homologous to the yeast *ScTPS2* and contains sequences corresponding to phosphatase and synthase domains (Paul et al., 2008; Delorge et al., 2014). Although most of the functions of Class II TPS is still ambiguous, many of the Class II genes appear to be extensively regulated at the transcriptional level, acting on abiotic stress (Bates et al., 2012; Tian et al., 2019; Usadel et al. 2008; Vishal et al. 2019). To explore the roles of Class II TPS members, several genes have been cloned and characterized in detail. In rice, overexpression of *OsTPS8* increased trehalose content, conferred salt stress tolerance and control related to plant yield (Vishal et al., 2019). TPS5 functions as a negative regulator of ABA signaling and is involved in altering the trehalose content and nitrate reductase activity (Tian et al., 2019). Very recently, a wheat TPS (*TaTPS11*) was reported to improve cold tolerance in arabidopsis without unfavorable phenotypes (Liu et al., 2019).

Tectona grandis Linn. f., popularly known as Teak (Lamiaceae family) is one of the most important wood sources due to its extraordinary qualities of color, density and durability, and is cultivated in the tropical regions with a significant market around the world (Galeano et al., 2015). Abiotic stress is an increasingly common and worrying phenomenon because it causes a loss of production in both agriculture and forestry. Although, teak is a tropical tree which needs alternating rainy and dry seasons to produce high-quality wood, planting teak in areas of water lack is of concern given their relatively high transpiration rates and reduced diameter growth (Sinacore et al., 2019).

Previous research has shown that after applying moderate and severe drought stress to teak seedlings, *TgTPS1* expression in control conditions was always lower than the expression of the plants submitted to moderate or severe water stress treatments, with a 2.5-fold increased expression in severe stress compared to control conditions (Galeano et al., 2019). These results led us to further investigate the role of *TgTPS1* in plant abiotic stress. In this research, the full-length coding

sequence (CDS) of *TgTPS1* was isolated from teak leaves and cloned in tobacco plants to investigate its role in the regulation of responses to drought and salt stress. In addition, we report the functional characterization of TgTPS1 and provide evidence for its important role in drought and salt stress response in plants.

3.2 CONCLUDING REMARKS AND FUTURE PROSPECTS

Given the importance of trehalose and the TPS family in the response to stress, here, we isolated and characterized a novel class II TPS protein from *T. grandis*. *TgTPS1* has been successfully isolated from teak and expressed constitutively in tobacco plants. The phenotype of *TgTPS1*-overexpressing plants was similar to the WT under control conditions, but the transgenic plants showed improved tolerance to salt and drought stress. The improved stress tolerance of transgenic plants was supported by morphological and physio-biochemical parameters, and activation of an array of stress-responsive genes at molecular level, leading to the synthesis of a wide range of protective compounds. Despite the precise mechanism by which trehalose acts on stress tolerance is far from being fully understood, the results presented here collaborates to establish a foundation for the study of TPS functions and its role on teak stress tolerance.

REFERENCES

- ACOSTA-MOTOS, J.R.; DÍAZ-VIVANCOS, P.; ÁLVAREZ, S.; FERNÁNDEZ-GARCÍA, N.; SÁNCHEZ-BLANCO, M.J.; HERNÁNDEZ, J.A. Physiological and biochemical mechanisms of the ornamental *Eugenia myrtifolia* L. plants for coping with NaCl stress and recovery. **Planta**, 242, 829–846, 2015.
- ALBACETE, A.; GHANEM, M. E.; MARTÍNEZ-ANDÚJAR, C.; ACOSTA M.; SÁNCHEZ-BRAVO, J.; MARTÍNEZ, V.; LUTTS, S.; DODD, I. C.; PÉREZ-ALFOCEA, F. Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (*Solanum lycopersicum* L.) plants. **Journal of Experimental Botany**, v. 59, n.15, p. 4119-4131, 2008.
- ALMEIDA, A.M.; VILLALOBOS, E.; ARA, J. M.; SANTO, D. M. Transformation of tobacco with an *Arabidopsis thaliana* gene involved in trehalose biosynthesis increases tolerance to several abiotic stresses. **Euphytica**, v.146, p. 165–176, 2005.
- ASHRAF, M.; FOOLAD, M.R. Roles of glycine betaine and proline in improving plant abiotic stress resistance. **Environment Experimental**, v. 59, p. 206–216, 2007.
- AVONCE, N.; LEYMAN, B.; MASCORRO-GALLARDO, J. O.; VAN DIJCK, P.; THEVELEIN, J. M.; ITURRIAGA, G. The *Arabidopsis* Trehalose-6-P Synthase *AtTPS1* gene is a regulator of glucose, abscisic acid, and stress signaling. **Plant Physiology**, v. 136, n. 3, p. 3649-3659, 2004.
- AVONCE, N.; MENDOZA-VARGAS, A.; MORETT, E.; ITURRIAGA, G. Insights on the evolution of trehalose biosynthesis. **BMC Evolutionary Biology**, v. 6, p. 109-123, 2006.
- BAENA-GONZALEZ, E.; ROLLAND, F.; THEVELEIN, J. M.; SHEEN, J. A central integrator of transcription networks in plant stress and energy signalling. **Nature**, v. 448, p. 938-942, 2007.
- BARTELS, D.; SUNKAR, R. Drought and salt tolerance in plants. *Critical Review Plant Science*, v. 24, p. 23–58, 2005.
- BAILEY, T.L.; JOHNSON, J.; GRANT, C.E.; NOBLE, W.S. The MEME suite. **Nucleic Acids Res**, 43, W39–W49, 2015.
- BATES, L. S.; WALDREN, R. P.; TEARE, I. D. Rapid determination of free proline for water-stress studies. **Plant Soil**, v. 39, p. 205–207, 1973.
- BATES, G. W.; ROSENTHAL, D. M.; SUN, J.; CHATTOPADHYAY, M.; PEFFER, E.; YANG, J.; ORT DR, J. A. M. A comparative study of the *Arabidopsis thaliana* guard-cell transcriptome and its modulation by sucrose. **Plos One** 7:e49641, 2012.
- BRASILEIRO, A. C. M.; CARNEIRO, V. T. C. **Manual de Transformação Genética de Plantas**. Brasília: Embrapa. 309p. 1998.

- CAPELLA-GUTIÉRREZ, S.; SILLA-MARTÍNEZ, J.M.; GABALDÓN, T. TRIMAL: A tool for automated alignment trimming in large-scale phylogenetic analyses. **Bioinformatics**, v. 25, p. 1972–1973, 2009.
- DELRORGE, I.; JANIÁK, M.; CARPENTIER, S.; VAN DIJCK, P. Fine tuning of trehalose biosynthesis and hydrolysis as novel tools for the generation of abiotic stress tolerant plants. *Frontiers Plant Science*, v. 5, p. 147, 2007.
- ELBEIN, A. D. The metabolism of α,α -trehalose. **Adv. Carbohydr. Chem. Biochem.** v. 30, p. 227–257, 1974.
- FAHAD, S.; BAJWA, A. A.; NAZIR, U.; ANJUM, S. A.; FAROOQ, A.; ZOHAIB, A. Crop Production under drought and heat stress: plant responses and management options. *Frontiers Plant Science*, v. 8, p. 1147, 2017.
- FIGUEROA, C. M.; FEIL, R.; ISHIHARA, H. Trehalose 6-phosphate coordinates organic and amino acid metabolism with carbon availability. **The Plant Journal**, v. 85, p. 410–423, 2016.
- FUJITA, M.; FUJITA, Y.; NOUTOSHI, Y.; TAKAHASHI, F.; NARUSAKA, Y.; YAMAGUCHI-SHINOZAKI, K. Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. **Currents Opinions Plant Biology**, v. 9, p. 436–42, 2006.
- GALEANO, E.; VASCONCELOS, T. S.; OLIVEIRA, P. N.; CARRER, H. Physiological and molecular responses to drought stress in teak (*Tectona grandis* L.f.). **Plos One**, v. 14, p. 1–26, 2019.
- GALEANO, E.; VASCONCELOS, T. S.; VIDAL, M.; MEJIA-GUERRA, M. K.; CARRER, H. Large-scale transcriptional profiling of lignified tissues in *Tectona grandis*. **BMC Plant Biology**, v. 15, 1, p.221, 2015.
- IJAZ, R.; EJAZ, J.; GAO, S.; LIU, T.; IMTIAZ, M.; YE, Z. WANG, T. Overexpression of annexin gene AnnSp2, enhances drought and salt tolerance through modulation of ABA synthesis and scavenging ROS in tomato. **Scientific Reports**, v. 7, p. 12087, 2017.
- JOSHI, R.; SAHOO, K. K.; SINGH, A. K.; ANWAR, K.; PUNDIR, P.; GAUTAM, R. K.; KRISHNAMURTHY, S. L.; SOPORY, S. K.; PAREEK, A.; SNEH LATA SINGLA-PAREEK, S. L. Enhancing trehalose biosynthesis improves yield potential in marker-free transgenic rice under drought, saline, and sodic conditions. **Journal of Experimental Botany**, v. 71, n. 2, p. 653–668, 2020.
- HARE, P.D.; CRESS, W.A.; VAN STADEN, J. Dissecting the roles of osmolyteaccumulation during stress. **Plant Cell Environ**, v. 21, p. 535–553, 1998.
- HARTHILL J.E.; MEEK S.E.; MORRICE N.; PEGGIE M.W.; BORCH J.; WONG B.H.; MACKINTOSH C. Phosphorylation and 14–3–3 binding of Arabidopsis trehalose-phosphate synthase 5 in response to 2-deoxyglucose. **Plant Journal**, v. 7, p. 211–223, 2006.
- HASSAN, A. L.; PACURAR, A.; LÓPEZ-GRESA, M. P.; DONAT-TORRES, M. P.; LLINARES, J. V.; BOSCAIU, M. Effects of salt stress on three ecologically distinct plantago species. **Plos one**, v. 11, n. 8, 2016.
- HU, X.; WU, Z-D.; LUO, Z-Y.; BURNER, D. M.; PAN, Y.B.; WU, C-W. Genome-Wide Analysis of the Trehalose-6-Phosphate Synthase (TPS) Gene Family and Expression Profiling of ScTPS Genes in Sugarcane. **Agronomy**, v. 10, p. 969, 2020.
- HUANG, X. S.; LIU, J-H.; CHEN, X. J. Overexpression of PtrABF gene, a bZIP transcription factor isolated from Poncirus trifoliata, enhances dehydration and drought tolerance in tobacco via scavenging ROS and modulating expression of stress-responsive genes. **BMC Plant Biology**, v.10, p. 230, 2010.
- HUNDERTMARK, M.; KINCHA, D. K. LEA (Late Embryogenesis Abundant) proteins and their encoding genes in Arabidopsis thaliana. **BMC Genomics**, v. 9, p. 118, 2008.
- KOSAR, F.; AKRAM, N. A.; SADIQ, M.; AL-QURAINY, F.; ASHRAF, M. Trehalose: a key organic osmolyte effectively involved in plant abiotic stress tolerance. **Journal of Plant Growth Regulation**, v. 38, p. 606–618, 2019.
- KOVACS, D.; KALMAR, E.; TOROK, Z.; TOMPA, P. Chaperone activity of ERD10 and ERD14, two disordered stress-related plant proteins. **Plant Physiology**, v. 147, p. 381–390, 2008.
- LAWSON, T.; LEFEBVRE, S.; BAKER, N. R.; MORISON, J. I. L.; RAINES, C. A. Reductions in mesophyll and guard cell photosynthesis impact on the control of stomatal responses to light and CO₂. **Journal of Experimental Botany**, v.59, n. 13, p. 3609–3619, 2008.
- LEYMAN, B.; VAN DIJCK, P.; THEVELEIN, J. M. An unexpected plethora oftrehalose biosynthesis genes in *Arabidopsis thaliana*. **Trends in Plant Science**, v. 6, p. 510–513, 2001.
- LI, H-W.; ZANG, B-S.; DENG, X-W.; WANG, X. P. Overexpression of the trehalose-6-phosphate synthase gene *OsTPS1* enhances abiotic stress tolerance in rice. **Planta**, v. 234, p. 1007–1018, 2011.
- LIN, Q.; YANG, J.; WANG, Q. Overexpression of the trehalose-6-phosphate phosphatase family gene *AtTPPF* improves the drought tolerance of *Arabidopsis thaliana*. **BMC Plant Biology**, v.19, n. 381, 2019.
- LIU, S. C.; JIN, J. Q.; MA, J. Q.; YAO, M. Z.; MA, C. L.; LI, C. F.; DING, Z. T.; CHEN, L. Transcriptomic analysis of tea plant responding to drought stress and recovery. **Plos One**, v. 11, 2016.
- LIU, X.; FU, L.; QIN, P.; SUN, Y.; LIU, J.; WANG, X. Overexpression of the wheat trehalose 6-phosphate synthase gene enhances cold tolerance in Arabidopsis thaliana. **Gene**, v. 710, p. 210–217, 2019.
- LIVAK, K. J.; SCHMITTGEN, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)). **Method. Methods**, v. 25, n. 4, p. 402–408, 2001.
- LUNN, J.E.; DELORGE, I.; FIGUEROA, C.M.; VAN DIJCK, P.; STITT, M. Trehalose metabolism in plants. **Plant Journal**, v. 79, 544–567, 2015.

- MAXWELL, K.; JOHNSON, G.N. Chlorophyll fluorescence: A practical guide. **Journal Experimental Botany**, v. 51, p. 659–668, 2000.
- MOSTOFA, M. G.; SERAJ, Z. I.; FUJITA, M. Exogenous sodium nitroprusside and glutathione alleviate copper toxicity by reducing copper uptake and oxidative damage in rice (*Oryza sativa* L.) seedlings. **Protoplasma**, v. 251, 1373–1386. 2014.
- OSUNA, D.; USADEL, B.; MORCUENDE, R.; GIBON, Y.; BLASING, O. E.; HOHNE, M.; GUNTER, M.; KAMLAGE, B.; TRETHEWEY, R.; SCHEIBLE, W.-R.; STITT, M. Temporal responses of transcripts, enzyme activities and metabolites after adding sucrose to carbon deprived Arabidopsis seedlings. **The Plant Journal**, v. 49, p. 463–491, 2007.
- PAUL, M. J.; PRIMAVESI, L. F.; JHURREEA, D.; ZHANG, Y. Trehalose metabolism and signaling. **Annual Review Plant Biology**, v. 59, p. 417–441, 2008.
- PAUL, M. Trehalose 6-phosphate. **Current Opinion Plant Biology**, v. 10, p. 303–309, 2007.
- NADEL, C. M.; MACKIE, T. D.; GARDNER, R. G. Osmolyte accumulation regulates the SUMOylation and inclusion dynamics of the prionogenic Cyc8-Tup1 transcription corepressor. **PLoS Genetics**, v. 15, 2019.
- PIECZYNSKI, M.; MARCZEWSKI, W.; HENNIG, J. Down-regulation of CBP80 gene expression as strategy to engineer a drought-tolerant potato. **Plant Biotechnol Journal**, v. 11, p. 459–469, 2013.
- RASHEED, S.; BASHIR, K.; MATSUI, A.; TANAKA, M.; SEKI, M. Transcriptomic analysis of soil-grown Arabidopsis thaliana roots and shoots in response to a drought stress. **Frontiers Plant Science**, v. 7, p. 180, 2016.
- ROMERO, C.; CUTANDA, M.C.; CORTINA, C.; CULIANEZ, M. F. A. Plant environmental stress response by trehalose biosynthesis. **Res. Trends Curr. Top. Plant Biol.**, v. 3, p. 73–88, 2003.
- ROMERO, C.; BELLE'S, J. M.; VAVA, J. L.; SERRANO, R.; CULIANEZ, M. F. A. Expression of the yeast trehalose-6-phosphate synthase gene in transgenic tobacco plants: pleiotropic phenotypes include drought tolerance. **Planta**, v. 201, p. 293–297, 1997.
- ROOSENS, N. H.; ALBITAR, F.; LOENDERS, K.; ANGENON, G.; JACOBS, M. Overexpression of ornithine-d-aminotransferase increases Probiogenesis and confers osmotolerance in transgenic plants. **Molecular Breeding**, v. 9, p. 73–80, 2002.
- SCHLUEPMANN, H.; BERKE, L.; SANCHEZ-PEREZ, G. F. Metabolism control over growth: a case for trehalose-6-phosphate in plants. **Journal Experimental Biology**, v. 63, n. 9, p. 3379–3390, 2012.
- SHABALA, S.; CUIN, T. A. Potassium transport and plant salt tolerance. **Physiologia Plantarum**, v. 133, p. 651–669, 2008.
- SINACORE, K.; BRETON, C.; ASBJORNSEN, HEIDI.; HERNANDEZ-SANTANA, VIRGINIA.; HALL, J. S. Drought effects on *Tectona grandis* water regulation are mediated by thinning, but the effects of thinning are temporary. **Frontiers in Forests and Global Change**, v. 2, p. 82, 2019.
- STILLER, I.; DULAI, S.; KONDRAK, M.; TARNAI, R.; SZABO, L.; TOLDI, O.; BANFALVI, Z. Effects of drought on water content and photosynthetic parameters in potato plants expressing the trehalose- 6-phosphate synthase gene of *Saccharomyces cerevisiae*. **Planta**, v. 227, n. 2, p. 299–308, 2008.
- STROM, A. R.; KAASEN, I. Trehalose metabolism in *Escherichia coli*: stress protection and stress regulation of gene expression. **Molecular Microbiology**, v. 8, p. 205–210, 1993.
- THIRUMALAIKUMAR, V.P.; DEVKAR, V.; MEHTEROV, N.; ALI, S.; OZGUR, R.; TURKAN, I. NAC transcription factor JUNGBRUNNEN1 enhances drought tolerance in tomato. **Plant Biotechnology**, v. 16, p. 354–366, 2017.
- TIAN, L.; XIE, Z.; LU, C.; HAO, X.; WU, S.; HUANG, Y.; LI, D.; CHEN, L. The trehalose-6-phosphate synthase TPS5 negatively regulates ABA signaling in Arabidopsis thaliana. **Plant Cell Reports**, v. 38, p. 869–882, 2019.
- TRISHLA V. S.; KIRTI, P. B. Structure-function relationship of *Gossypium 1 hirsutum* NAC transcription factor, GhNAC4 with regard to ABA and abiotic stress responses
- USADEL, B.; BLASING, O. E.; GIBON, Y.; RETZLAFF, K.; HOHNE, M.; GUNTHER, M.; STITT, M. Global transcript levels respond to small changes of the carbon status during progressive exhaustion of carbohydrates in *Arabidopsis* rosettes. **Plant Physiology**, v. 146, p. 1834–1861, 2008.
- VANDESTEENE, L.; MATTHEW, R.; ROY, K.; VAN DIJCK, P.; ROLLAND, F. A Single Active Trehalose-6-P Synthase (TPS) and a Family of Putative Regulatory TPS-Like Proteins in Arabidopsis. **Molecular Plant**, v. 3, n.2, p. 406–419, 2010.
- VAN HOUTTE, H.; VANDESTEENE, L.; LOPEZ-GALVIS, L.; LEMMENS, L.; KISSEL, E.; CARPENTIER, S.; FEIL, R.; AVONCE, N.; BEECKMAN, T.; LUNN, J. E.; VAN DIJCK, P. Overexpression of the trehalase gene *AtTRE1* leads to increased drought stress tolerance in *Arabidopsis* and is involved in abscisic acid-induced stomatal closure. **Plant Physiology**, v. 161, p. 1158–1171, 2013.
- VISHAL, B.; KRISHNAMURTHY, P.; RAMAMOORTHY, R.; KUMAR, P. P. OsTPS8 controls yield-related traits and confers salt stress tolerance in rice by enhancing suberin deposition. **New Phytology**, v. 221, p. 1369–1386, 2019.
- WANG, W. B.; KIM, Y. H.; LEE, H. S.; KIM, K. Y.; DENG, X. P.; KWAK, S. S. Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. **Plant Physiol Biochem**, v. 47, p. 570–577, 2009.

- WIEMKEN, A. Trehalose in yeast, stress protectant rather than reserve carbohydrate. **Antonie Van Leeuwenhoek**, v. 58, p. 209–217, 1990.
- XIA Z.; SU, X.; LIU, J.; WANG, M. The RING-H2 finger gene 1 (RHF1) encodes an E3 ubiquitin ligase and participates in drought stress response in *Nicotiana tabacum*. **Genetica**, v. 141, p. 11–21, 2013.
- XUAN, J.; FENG, Y.; WENG, M.; GE, Z.; Expressed sequence tag analysis and cloning of trehalose-6-phosphate synthase gene from marine alga *Laminaria japonica* (Phaeophyta). **Acta Oceanologica Sinica**, v. 31, n.6, 2012.
- ZENTELLA, R.; MASCORRO-GALLARDO, J. O.; VAN DIJCK, P.; FOLCH-MALLOL, J.; BONINI, B.; VAN VAECK, C.; GAXIOLA, R.; COVARRUBIAS, A. A.; NIETO-SOTELO, J.; THEVELEIN, J. M. Selaginella lepidophylla trehalose-6-phosphate synthase complements growth and stress-tolerance defects in a yeast tps1 mutant. **Plant Physiology**, v. 119, n. 4, p. 1473–1482, 1999.
- ZHANG, B.; SU, L.; HU, B.; LI, L. Expression of AhDREB1, an AP2/ERF Transcription Factor Gene from Peanut, Is Affected by Histone Acetylation and Increases Absciscic Acid Sensitivity and Tolerance to Osmotic Stress in Arabidopsis. **International Journal Molecular Science**, v. 19, n.5, p. 1441, 2018.
- ZHANG, Z.; LI, J.; LI, F.; LIU, H.; YANG, W.; CHONG, K.; XU, Y. OsMAPK3 phosphorylates OsbHLH002/OsICE1 and inhibits its ubiquitination to activate OsTPP1 and enhances rice chilling tolerance. **Developmental Cell**, v. 43, p. 731–743, 2017.
- ZHANG, S.Z.; YANG, B. P.; FENG, C. L.; CHEN, R. K.; LUO, J. P.; CAI, W. W.; LIU, F. H. Expression of the *Grifola frondosa* trehalose synthase gene and improvement of drought-tolerance in sugarcane (*Saccharum officinarum* L.). **Journal Integral Plant Biology**, v.48, n. 4, p. 453–459, 2006.
- ZHANG, S. Z.; YANG, B. P.; FENG, C. L.; TANG, H. L. Genetic transformation of tobacco with the trehalose synthase gene from *Grifola frondosa* Fr. enhances the resistance to drought and salt in tobacco. **Journal Integral Plant Biology**, v. 47, n. 5, p. 579–587, 2005.
- ZHU, J.K. Salt and drought stress signal transduction in plants. **Annual Review Plant Biology**, v. 53, p. 247–273, 2002.

GENERAL CONCLUSIONS

The present work is one of the first study of functional characterization of teak genes. In this research, functional studies were conducted to characterize the three selected genes, TgMYB2, TgERF1 and TgTPS1. TgMYB2 protein, a new 3R-MYB transcription factor, is located in the nucleus, confirmed by stable and constitutive expression in tobacco. Transgenic tobacco plants showed increased biomass compared to WT plants, supported by morphological and physiological parameters, through a coordinated regulation of cellulose, hemicellulose and lignin biosynthesis. The gene TgERF1, which encodes an AP2/ERF transcription factor has been successfully isolated from *T. grandis*, and expression of TgERF1 was rapidly induced by Polyethylene Glycol (PEG), NaCl, and exogenous ABA, ACC and JA treatment, suggesting a potential role in drought and salt tolerance in teak. TgERF1 exhibited an exclusive expression in the nucleus, and does not present transmembrane motifs, which is in accordance with the role of its protein as a transcription factor. The 35S::TgERF1 transgenic lines presented had a significant tolerance response under drought and salt stress, showing altering physio-biochemical parameters, and activation of an array of stress-responsive genes at molecular level. Dada a importância da trealose e da família TPS na resposta ao estresse, aqui foi isolada e caracterizada uma nova proteína TPS de classe II de *T. grandis*. In this work, the full-length coding sequence (CDS) of TgTPS1 was isolated from teak leaves and cloned in tobacco plants to investigate its role in the regulation of responses to drought and salt stress. Here, the functional characterization of TgTPS1 has been reported and provided evidence for its important role in drought and salt stress response in tobacco. The data obtained contribute to understanding of the molecular mechanism in woody plants and tolerance for environmental variations, can be used in applied and basic science along to biotechnological approaches.