Teak Seedlings' Physiological and Gene Expression Responses to Salt and Osmotic Stress

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Abstract

Medicinal and Aromatic Plants Research Station and Department of Agricultural Biotechnology, Anand Agricultural University, Gujarat, conducted the experiment in May 2019 to determine the physiological and differential gene expression analysis of teak seedlings under various abiotic stress conditions (control, 150 mM NaCl and 15% PEG). The physiological data [chlorophyll content, membrane stability index and relative water content] were recorded at 0, 2, 7, and 12 DAT with four repetitions. These parameters were all lowered quantitatively at first, and then considerably during longer treatment. The application of 150 mM NaCl has disastrous effects on plant physiology in terms of PEG. The findings revealed that diverse stresses have a substantial impact on seedling physiology due to chlorophyll degradation, cell and chloroplast membrane damage, ROS formation, and decreased water absorption in response to physiological or physical shortage of accessible soil moisture. At 12 DAT, the gene expression profile of treated seedlings was compared to that of control seedlings. RT-PCR was used to examine the expression of one endogenous and ten stress-related genes. MYB-3, HSP-1, BI-1, and CS-2 genes were up-regulated in leaves of stress-treated seedlings. The genes’ up-regulation supported their protective role in plants under abiotic stress. Treatments, stress duration, and plant species all altered the expression profile of genes. According to the findings of this study, these physiological indicators could be used as marker indices to measure tree’s stress tolerance capacity during the seedling stage. The up-regulated genes will be further investigated and used to confirm stress resistance and susceptible teak seedlings.

Introduction

The establishment, growth, and survival of seedlings are critical processes in forest development. Light and water are key factors in the growth and survival of seedlings and tree regeneration in tropical forests (Cavin et al., 2013). According to climate change forecasts, the intensity and frequency of abiotic stress would increase in the near future for the majority of tropical forests (Amissah et al., 2015). The composition and distribution of species in forest conditions are determined by abiotic stress resistant seedlings. Teak (Tectona grandis L.) is a tropical tree that requires a rainy and dry season cycle to produce high-quality wood (Cantino et al., 1998). The teak tree is well-known for its use in furniture and cabinetry (Gill et al., 1983). The weather conditions in Gujarat’s south region, Gir and Girnar, are more conducive to teak growing (Singh, 2013).

Because of their sessile nature, plants are unable to relocate to avoid stress. They do, however, have distinct morphological, physiological, and molecular strategies that enable plants survive in stressful situations (Al-Whaibi, 2011). Abiotic circumstances cause stress signal transduction and diminish plant biosynthetic power, with disastrous consequences (Hamanishi & Campbell, 2011). The perception of stress signals and the activation of complicated signaling pathways result in significant changes in gene expression, which is a prerequisite for acclimatization (Rosero et al., 2011). Abiotic stress, such as salt and osmotic stress, causes a decrease in water flux, stomatal closure, and carbon dioxide fixation, as well as an increase in reactive oxygen species (ROS). Increased levels of reactive oxygen species (ROS)
change plant physiology by oxidizing cellular components, which destroys cellular membranes and leads to cell death (Das & Roychoudhury, 2014 & Wang, 2014).

The greenness of a leaf is measured by chlorophyll. Photo-oxidation, rupture of the chloroplast and thylakoid membranes, disintegration of chlorophyll molecules, and defective chlorophyll production all diminish chlorophyll levels under abiotic stress conditions (Jafarnia et al., 2017; Lu et al., 2021 & Abdolinejad & Shekafandeh, 2022). To detect stress injury to the cell membrane, changes in electrical impedance and electrolyte leakage were evaluated. Leakage is proportional to the membrane's ability to absorb and retain solutes, and so reflects changes in membrane potentials and permeability caused by stress (Rahman et al., 2016; Jafarnia et al., 2017 & Tafeshri et al., 2021). The degree of hydration of cells and tissues is determined by relative water content, which also represents the physiological functioning and growth activities of plants (Sinhababu & Banerjee, 2013). The water potential of leaves reflects soil moisture levels. The two basic factors influencing water migration to new cell division sites are water content and water potential (Karimi et al., 2012; Rad et al., 2021 & Tafeshri et al., 2021).

Plants employ a number of complicated regulatory systems to counteract the effects of abiotic stress, including increased or repressed gene expression and intricate cross-talk between biochemical and molecular processes (Sánchez-Fernández et al., 1997). Through the up and down regulation of corresponding genes in response to environmental and developmental factors, we can examine complicated biological processes such as signal transduction pathways and metabolic pathways. Because of its precision, reliability, sharpness, and reproducibility, RT-PCR is commonly employed for gene expression analysis (Bustin, 2002). For accurate and reliable gene expression analysis using reference genes, RT-PCR data must be normalised (Reboucas et al., 2013). The key reference genes GAPDH, β-actin, 18S, and 28S rRNAs are commonly employed as reference genes (Suzuki et al., 2000). The threshold cycle (CT) is the cycle in which the fluorescence level reaches a specific level that may be measured by a detector. As a result, CT value is directly employed as a normalizer to compute relative gene expression in treated and control samples (Rao et al., 2013).

MYB TFs have a DNA binding domain (Liu et al., 2017) and are most prevalent in plants (Schwechheimer & Bevan, 1998 & Peng et al., 2016). It plays a role in cell cycle control, hormone signaling, secondary metabolism, and abiotic stress responses (Cao et al., 2013 & Baillo et al., 2019). Heat shock proteins aid in plant growth, development, and abiotic stress response, as well as preventing protein misfolding (Lee et al., 1997). BAX is a mitochondrial membrane protein that disrupts plant and yeast biological systems, resulting in cell death (Kawai et al., 1999; Danial and Korsmeyer, 2004). BAX inhibitor-1 (BI-1) is an ER protein that plays a role in plant defence and prevents Bax-induced cell death (Kawai et al., 1999). Carboxylesterases (CXEs) play a role in HR in plants by synthesising or degrading signalling molecules in response to stress (Gershater and Edwards, 2007). Several stress hormones accumulate as carboxylesters and amides, which are activated by carboxylesterases, salicylic acid, and other enzymes (Kumar & Klessig, 2003) and jasmonic acid (Stuhlfelder et al., 2004). When organisms are exposed to environmental stress, phenylalenine ammonia lyase (PAL) is involved in the manufacture of precursors for numerous secondary metabolites (Huang et al., 2010 & Richard et al., 2011). The plasma membrane,
which contains catalytic cellulose synthase subunits, is where cellulose production takes place (Roelofsen, 1958; Mueller and Brown, 1980 & Kimura et al., 1999). Under abiotic stress, the cellulose content XTH and expansions are reduced due to changes in cellulose biosynthesis (Gall et al., 2015) and the formation of reactive oxygen species (ROS) (Tenhaken, 2014).

Agro foresters are currently researching cellular mechanisms that aid in the greater survival of forest trees under abiotic stress. The findings would be utilized to design methods for forest tree growth and survival in the face of this specific environmental danger (Hamanishi & Campbell, 2011). In this regard, the experiment was designed to look at the most important physiological and gene expression characteristics in teak seedlings that are affected by abiotic stress. The findings of this study will, in general, provide basic knowledge about the teak tolerance mechanism under various abiotic stress conditions.

**Materials And Methods**

**Experimental Samples**

During the month of May 2019, 150 young, healthy, comparable, and six-month-old teak seedlings were obtained from Clonal nursery farm, Mogar NH-8, Mogar, Anand, Gujarat, India-388340 (22°31’29.7"N 73°00’49.4"E). The seedlings were moved to the green house of Anand Agricultural University's Medicinal and Aromatic Plants Research Station, Anand, Gujarat, India-388110 (22°53’65.8"N 72°98’22.8"E). All seedlings were watered until they were completely saturated; around 20 ml of water was enough to wet the entire little seedling bags (4X6 cm). The seedlings were kept in the green house for one week to adjust to their new surroundings and get the necessary irrigation. Seedlings were adapted and ready for experimentation after one week. The experiment was designed in this fashion, with four repetitions in Completely Randomized Design (CRD).

The 120 teak seedlings that survived and were healthy were chosen and divided into three groups, each with 40 plants. Each group received one treatment, which was subsequently subdivided into four subgroups (i.e., four repetitions) of ten plants each. The seedlings were irrigated with tap water in the morning on the first day, and then they were ready for the treatments. In the evening, the seedlings were given one of three treatment solutions: control (T₁), 150 mM NaCl (T₂), or 15% Polyethylene glycol (PEG) (T₃). After 7 days, the treatments were repeated, and the control plants were irrigated with tap water as needed.

**Physiological Examination**

By morphological appearance, stress-affected seedlings were judged as wilted. In addition, the effect of abiotic stress on teak seedlings was investigated using several physiological markers. Physiological characteristics such as chlorophyll concentration, membrane stability index, and relative water content were measured. At 0, 2, 7, and 12 days after treatment, promising wilting leaf samples of teak seedlings from treatments and healthy leaf samples from the control were obtained for physiological investigation.
A. Chlorophyll Content (SPAD value): The chlorophyll content of a fully expanded leaf sample was determined with a SPAD meter (502 Minolta Co., Japan) and expressed as a SPAD value (Mielke et al., 2010).

B. Membrane Stability Index (MSI): The leaf membrane stability index was determined using the method of Premchandra et al. (1990). With distilled deionized water, the leaves were carefully cleaned. To remove surface wetness, the leaves were cleaned with tissue paper. With a sharp blade, the leaves were cut into 5 mm² leaf discs. About 20 leaf discs were soaked in 30 ml distilled deionized water in a clean test tube. For one hour, these tubes were warmed in a 37°C water bath. After cooling the tubes to room temperature, the initial electrical conductivity (EC₁) was measured with the Multi-Parameter PCS Testr™ 35 equipment. The test tubes were placed in a 100°C water bath for another 10 minutes. The tubes were cooled to ambient temperature before being tested for electrical conductivity (EC₂). The MSI was determined using the formula below.

\[ MSI = 1 - \frac{EC_1}{EC_2} \times 100 \]

C. Relative Water Content (RWC): The leaf samples were promptly weighed for RWC to get fresh weight (FW). The leaves were placed in petri plates with enough distilled water to become fully turgid in a dark environment at 4°C for 24 hours to check transpiration and photosynthesis and avoid dry weight loss. The leaves were weighed the next day after being cleansed with blotting paper to remove surface water (TW). The leaves were dried in a hot air oven at 100°C until they reached a steady weight, and then weighed (DW). The relative water content was calculated using Morgan's (1984) equation and given as a percentage.

\[ RWC = \frac{FW-DW}{TW-DW} \times 100 \]

Analytical Statistics

The statistical analysis of the physiological data collected during the inquiry was performed using software according to Panse and Sukhatme's approach (1978).

**RNA extraction from leaf samples and cDNA synthesis:**

Five medium-sized wilted leaf samples were obtained from control and stress-treated seedlings and found to be promising. In May of this year, sampling was done in the afternoon from 2:00 pm to 4:00 pm. The leaf samples were washed with distilled water and then wiped with fresh tissue paper. After wiping, the leaf was sprayed with RNA Zap on both sides and wiped with sterilized tissue paper again. RNA Zap wiped scissor was used to cut the leaf petiole. The cut samples were kept in RNA Later. After that, the vials were placed in a sample collection box having ice flakes. For further study, these materials were frozen at -80°C.
The total RNA from leaf tissues was extracted using the Qiagen RNeasy Plant Mini Kit (Qiagen cat, 74104) according to the manufacturer's instructions. The Qubit® 3.0 Fluorometer and the Agilent Bioanalyzer 2100 were used to assess the quality, quantity, and integrity of total RNA. For downstream applications, RNA samples having an A260/280 ratio of 2 to 2.2 and a RIN (RNA Integrity Number) of 6 to 9 were processed. Every RNA sample that exceeded the quality control standards had its cDNA synthesized in duplicate. With the use of the PrimeScript RTase kit, 1.5 µg of total RNA was utilized to make cDNA (TAKARA cat, 6110A).

**Real-Time PCR for Gene Expression Analysis:**

CFX-96 Real-Time PCR was used to investigate differential gene expression (BioRad). The current study analyzed a total of ten stress sensitive genes acquired from earlier research by various scientists (Supplementary Table S1). All PCR reactions were carried out in 96-well MicroAmpTM Optical 96-Well Reaction Plate with Barcode plates. Primscript SYBR green premix (TAKARA) containing 10 pmol of each gene specific forward and reverse primer and 2 µl of cDNA template was used for amplification in a final reaction volume of 20 µl. In triplicate, RT-PCR of endogenous and abiotic stress responsive genes was performed with a non-template control (NTC). The following were the RT-PCR conditions: In 96-well optical reaction plates, 94°C for 30 s was followed by 44 cycles of 94°C for 12 s, 60°C for 30 s, 72°C for 40 s, and 81°C for 1 s. The fold change in expression was estimated using the average of three replication Ct cycles. CFX Manager version 3.1 was used to analyze data from real-time PCR (Biorad).

**Results**

The physiological parameters chlorophyll content, relative water content, and membrane stability index were examined and data was recorded at 0, 2, 7, and 12 DAT to validate the induction of abiotic stress in teak seedlings. Table 1 shows the results of statistical analysis for various treatments, while Fig. 1 shows photos of treated seedlings at various time intervals.
### Table 1
Effects of various abiotic stress producing treatments on teak seedling physiological parameters

<table>
<thead>
<tr>
<th>Physiological Parameters</th>
<th>Days after treatments (DAT)</th>
<th>Treatments</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>150 mM NaCl</td>
</tr>
<tr>
<td>Chlorophyll content (SPAD Value)</td>
<td>0</td>
<td>49.05</td>
<td>48.74</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>44.86</td>
<td>44.57</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>46.85</td>
<td>40.71</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>45.83</td>
<td>35.14</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>46.65</td>
<td>42.29</td>
</tr>
<tr>
<td>Membrane Stability Index (MSI)</td>
<td>0</td>
<td>81.22</td>
<td>82.17</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>79.83</td>
<td>77.17</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>78.34</td>
<td>73.97</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>77.45</td>
<td>68.70</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>79.21</td>
<td>75.50</td>
</tr>
<tr>
<td>Relative Water Content (%)</td>
<td>0</td>
<td>86.57</td>
<td>88.00</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>84.39</td>
<td>79.90</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>82.96</td>
<td>74.03</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>82.34</td>
<td>72.23</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>84.06</td>
<td>78.54</td>
</tr>
</tbody>
</table>

**Chlorophyll content (SPAD value)**

The chlorophyll content of stress-treated seedlings was gradually lowered numerically till 12 DAT. Initially, there was no significant loss in chlorophyll content in any treatments; however it was significantly reduced at 7 DAT (Table 1). From 0 to 12 DAT, the chlorophyll content of seedlings treated with 150 mM NaCl was lowered from 48.74 to 35.14 SPAD value. The chlorophyll concentration was similarly lowered from 48.12 to 39.73 SPAD value when 15% PEG was used. The chlorophyll content of control seedlings was found to be 46.65 SPAD on average (Fig. 2).

**Membrane stability index (MSI)**

During the experiment, the MSI of control seedlings was found to be on average 79.21 (Table 1). In stress-treated seedlings, the MSI dropped consecutively. There was no substantial reduction in MSI at first. However, after 2 DAT, the control (79.83) had a considerably higher MSI than the 15% PEG treatment.
(79.79), and identical data was observed at 7 DAT in both the control and 15% PEG. The 150 mM NaCl treatment (73.97) had the lowest MSI at 7 DAT, followed by 15% PEG (77.11). In comparison to control (77.45), MSI was considerably reduced in 150 mM NaCl (68.70) followed by 15% PEG (73.59) treatments at 12 DAT (Fig. 3).

Relative water content (RWC)

In terms of the RWC, there were no significant changes between the treatments at 0 and 2 DAT (Table 1). However, at 2 DAT, untreated seedlings had the highest RWC (84.39%), followed by 15% PEG (83.46) and 150 mM NaCl treatment (79.90%). At 7 DAT, there was a significant difference in RWC, with the control seedlings having the highest RWC (82.96%) and the 150 mM NaCl treated seedlings having the lowest (74.03%). RWC was found to be higher in control seedlings (82.34%), 15% PEG (76.14%), and lowest in 150 mM NaCl (72.23%) at 12 DAT, which was statistically significant (Fig. 4).

Gene Expression Analysis in Real Time:

RT-PCR was used to examine the expression of an abiotic stress responsive gene. Six abiotic stress sensitive genes, including MYB-3, HSP-1, BI-1, CESs, PAL-1, and CS-2, as well as the endogenous gene Actin 2/7, demonstrated robust amplification with cDNA from control and treated samples. In all treatment groups, the fluorescence from the amplicon was greater than the background fluorescence at Ct values of 29.86, 25.81, 30.94, 27.20, 34.05, and 32.46, respectively, in MYB-3, HSP-1, BI-1, CESs, PAL-1, and CS-2 genes (Table 2). Single peak in melt curve analysis was represented in Supplementary Fig. S1 that indicated only gene specific sequence was amplified without any primer-dimer and non specific amplification of DNA (Table 2). Moreover, non-template control did not show any amplification this indicated that samples and reagents were not contaminated as well as did not have any handling error.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cycle Threshold (Ct) Value</th>
<th>Actin2/7</th>
<th>MYB-3</th>
<th>HSP-1</th>
<th>BI-1</th>
<th>CESs</th>
<th>PAL-1</th>
<th>CS-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>35.13</td>
<td>30.26</td>
<td>28.26</td>
<td>34.42</td>
<td>28.20</td>
<td>34.57</td>
<td>36.40</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>29.53</td>
<td>30.73</td>
<td>25.56</td>
<td>29.78</td>
<td>27.09</td>
<td>35.17</td>
<td>33.98</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>33.10</td>
<td>28.58</td>
<td>23.60</td>
<td>28.61</td>
<td>26.31</td>
<td>32.41</td>
<td>27.01</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>32.59</td>
<td>29.86</td>
<td>25.81</td>
<td>30.94</td>
<td>27.20</td>
<td>34.05</td>
<td>32.46</td>
<td></td>
</tr>
<tr>
<td>Tm</td>
<td>81.50</td>
<td>82.00</td>
<td>87.00</td>
<td>80.00</td>
<td>85.00</td>
<td>77.50</td>
<td>83.50</td>
<td></td>
</tr>
</tbody>
</table>

Housekeeping genes like, GAPDH, β-actin, 18S and 28S rRNAs are commonly employed as reference genes (Suzuki et al., 2000). As a result, the Actin gene was used as a reference gene in the current investigation. To compensate for inter and intra-kinetic RT-PCR variance, target gene expression was
normalised using Actin 2/7 as an endogenous control. Supplementary Fig. S1 shows the amplification and melt curves for all of the genes employed, and Table 3 shows the relative quantity.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Relative Quantity (RQ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MYB-3</td>
</tr>
<tr>
<td>T₁</td>
<td>1.00</td>
</tr>
<tr>
<td>T₂</td>
<td>1.12</td>
</tr>
<tr>
<td>T₃</td>
<td>0.79</td>
</tr>
</tbody>
</table>

The MYB gene product is a key transcription factor for several stress-related genes, including hormone signaling and abiotic stress tolerance. As a result, under stress, MYB gene expression would be up-regulated, enhancing the expression of other stress-related genes. In comparison to control seedlings, the MYB-3 gene was considerably up-regulated (1.12). In PEG treatment, however, MYB-3 gene expression was significantly reduced (0.79). Under stress, the HSP protein serves as a molecular chaperon, protecting proteins from misfolding. The HSP-1 gene was considerably up-regulated (6.20) in 15% PEG stress compared to control in the current study, whereas it was down-regulated in salt stress (0.13). The BAX inhibitor gene produces a cryoprotective protein that is found in the endoplasmic reticulum (ER) membrane and prevents cell death in stressful situations. Surprisingly, the apoptosis-related gene BL-1 was up-regulated the most in 15% PEG (13.72) when compared to control and 150 mM NaCl (0.51). When compared to control, the expression of CESs was observed to be down-regulated in 150 mM NaCl (0.045) and 15% PEG (0.911) treatments. In comparison to control, RQ analysis revealed down-regulation of the PAL-1 gene in 150 mM NaCl (0.017) and 15% PEG (0.014) treatments. The degree to which this gene is suppressed varies from root to leaf. The expression level of the CS-2 gene was up-regulated (2.14) in 15% PEG, control (1.00), but down-regulated in 150 mM NaCl (0.14) treatments, according to RQ analysis. The degree to which this gene is suppressed varies from root to leaf.

**Discussion**

The study of the diverse physiological and molecular mechanisms of plant seedlings under various abiotic stresses could be an appealing issue for scientists looking to generate resistant lines. These abiotic stress-resistant plant seedlings will considerably outperform future abiotic stress challenges in orchard preparation and forestation. Plants underwent morphological, physiological, and cellular changes as a result of abiotic stress. We investigated the impact of abiotic stress on three key physiological characteristics that determine plant growth, survival, and productivity in this experiment. The expression of stress sensitive genes was also examined in order to confirm their expression pattern under stress.

**Physiological Analysis:**
When compared to control seedlings, salt treated seedlings had lower chlorophyll concentration followed by osmotic stress. Lu et al. (2021) found a similar finding in three woody plant species (*Tamarix ramosissima, Populus euphratica,* and *Haloxylon ammodendron*) cultivated under various levels of saline stress. *P. euphratica* reduced Chl more than *H. ammodendron* and *T. ramosissima* at lower NaCl concentrations. Under BS cultivars, chl a, chl b, and Tchl were considerably reduced in moderate and high salinity stress (Rahneshan, et al., 2018) also in the natural forest trees of Eastern China's North-South Transect (Li et al., 2018). With increasing levels of PEG-induced water stress, photosynthetic pigment was shown to be significantly reduced in the fig genotypes at 15% PEG treatment (Abdolinejad & Shekafandeh, 2022) and in leaves of the four tree species *B. variegate, C. fistula, D. regia* and *P. pterocarpum* (Sinhababu & Banerjee, 2013). Photo-oxidation, chlorophyll breakdown by the enzyme chlorophyllase, and reduced chlorophyll production are the main causes (Santos, 2004 & Jafarnia et al., 2017). High levels of sodium (Na+) and chloride (Cl-) ions in chloroplasts cause considerable damage to the thylakoid membrane, (Wu and Zou, 2009) resulting in chlorophyll leaching and thylakoid degeneration (Sneha et al., 2012). The pigment-protein-lipid complex may be weakened in high-saline conditions (Levitt 1980), and salinity may promote the activity of the Chl-degrading enzyme chlorophyllase (Reddy and Vora 1986). Salinity stress reduces chlorophyll content by depleting Mg2+ in plants, which is a critical component of chlorophyll (Khan et al. 2000). Furthermore, ROS are abundantly formed in plants due to irreversible photophosphorylation inactivation and electron transport obstruction in the thylakoid membrane. Degrading the chloroplast and activating anti-oxidative scavenging systems to safeguard cell structure can reduce ROS generation (Veiga et al., 2007 & Mittal et al., 2012). Plant species, duration, severity, and amount of stress all influence chlorophyll decrease under stress.

Membrane stability index is a measurement of membrane integrity and permeability. Cell membrane integrity or electrolyte leakage reflects cell health in relation to external conditions and is extensively used as a reliable indication of plants under abiotic stress (Fan & Blake, 1997 & Tafeshri et al., 2021). MSI was dramatically reduced in salt and osmotic stress seedlings at 12 DAT when compared to control irrigated seedlings. When comparing control and enhanced salt stress, our findings show lower membrane stability in *Acacia auriculiformis* (Rahman et al., 2016). Yue et al. (2018) found that salinity stress reduced MSI by 31.7 percent to 54.1 percent at 100 and 200 mM in black locusts (*Robinia pseudoacacia*). Pistachio rootstock treated with 100 to 200 mM NaCl showed a considerable reduction in MSI (Rad et al., 2021). The findings are similarly consistent with those of PEG-treated almonds (*Prunus dulcis* (Mill.)), which had lower MSI as PEG concentration increased (Karimi et al., 2012). At 10% PEG, fig (*Ficus carica*) explants showed increased ion leakage, but tetraploid explants showed larger ion leakage at 20–25% PEG treatment (Abdolinejad & Shekafandeh, 2022). In cells, a lack of water causes an imbalance in metabolism and the generation of reactive oxygen species (ROS) (Karimi et al., 2012). These free radicals are the primary cause of cell membrane damage. All components bound in the cell and organelles are released when the cell membrane is disrupted (Abdolinejad & Shekafandeh, 2022). Ions primarily flow out of the cell, and the number of ions released is a measure of the cell membrane's integrity. Ion leakage is associated with decreased cell membrane stability. As a result, under drought stress, membrane stability
is compromised by numerous defects (Cao et al., 2017 & Jafarnia et al., 2017), and electrolytes are lost to the environment.

In plants, relative water content (RWC) is an important and dependable indicator of a cell’s ability to retain water under unfavorable conditions. It establishes a balance between the water supply to the plant system and the rate of transpiration through the stomata (Sinhababu & Banerjee, 2013). When compared to control, RWC was dramatically lowered in 150 mM NaCl followed by 15% PEG at 7 DAT. Yue et al. (2013) found similar low RWC data in *Robinia pseudoacacia* under 100 and 200 mM NaCl stress, as well as in *Pistacia vera* var. Ghermez-Pesteh under 200 mM NaCl stress (Rad et al., 2021). Tafeshri et al. (2021) found that 3% PEG and 6% PEG reduced RWC in *Myrtus communis* shoots. RWC in almond explant leaves was reduced when the PEG concentration in the media was increased (Karimi et al., 2012). Sinhababu & Banerjee (2013) found that PEG treatment reduced RWC in *C. fistula* and *B. variegate* compared to control. RWC is involved in absorbing more water from the soil and/or controlling water loss through the stomata (Xiao et al., 2009 & Cao et al., 2017). The characteristics and structure of cells are altered under abiotic drought stress, and they are unable to maintain turgor and osmotic pressure (Bolat et al., 2014). The osmotic pressure of soil water is higher than that of plant root cells due to the larger concentration of dissolved salts. As a result, plant root cells are unable to receive water from the soil via osmosis (Nejadsahebi et al., 2010 & Zarafshar et al., 2014). Furthermore, there is no other available type of water for absorption by plant root cells when water is scarce (Ying et al., 2015 & Haider et al., 2018).

**Gene Expression Analysis:**

MYB transcription factors are found throughout higher plants and are involved in abiotic stress responses (Liu et al., 2017). *MYB-3* was shown to be considerably up-regulated by salt stress as compared to control. Cao et al. (2013) found that genes including MdoMYB22, 121, 146, 148, 155, and 206 displayed up-regulations in Arabidopsis under NaCl and PEG stress conditions. Under salt and PEG treatment, the MYB genes Aco001113 and Aco007733 were likewise shown to be up-regulated in pineapple (Liu et al., 2017). Similarly, during NaCl and PEG treatments, the expression of MYB genes was up-regulated in *Tamarix hispida*, a woody plant (Zhang et al., 2018). Through RT-PCR, eight MYB genes were up-regulated for NaCl stress and eight MYB genes were down-regulated for PEG6000 stress in *T. mongolica*. Their findings varied depending on the duration of stress and the tissue (Chen et al., 2019). Under 300 mmol/l NaCl treatment, oil palm revealed greater *EgMYB* gene expression at various time intervals (Zhou, et al., 2020). *JrMYB73* expression was up-regulated in walnut following 20% polyethylene glycol stress, but *JrMYB44* expression was down-regulated. When comparing *JrMYB44* 3 h to control, the transcription value decreased (Li et al., 2021). Based on our findings and previous research, we believe that the teak *MYB-3* gene regulates secondary cell wall deposition, cell cycle control, hormone signaling, secondary metabolism, meristem development, and cellular morphogenesis under abiotic stress conditions (Zhang et al., 2018 & Yang et al., 2019). As a result, increased expression of multiple *MYB* genes improves many species’ ability to withstand abiotic stress (Li et al., 2021). Furthermore, the structure and function of MYB TF family genes vary substantially between species (Yang et al., 2019).
In the current study, 15% PEG stress increased HSP expression. According to Robinet et al. (2010), expression of HSPs in loblolly pine was co-regulated with photosynthetic acclimation under mild drought and was differently regulated based on different water regimes. The divergent expression patterns of Cqhsp70s in quinoa during drought were also reported by Liu et al. (2018), showing the various functions of HSPs genes in drought tolerance and the functional diversity of Hsp70. HSPs are also known to operate as molecular chaperones, limiting the aggregation formation of other proteins, as well as participating in protein folding and mending misfolded conformers in stressed or unstressed cells (Robinet et al., 2010; Zhang et al., 2015; Lui et al., 2018 & Yer et al., 2018). SHSP expression has risen slightly in response to heat, salt, oxidative, and water stress. As a result, HSP protein expression is one of the most important indicators of persistent stress (Sun et al. 2002 & Zhai et al., 2016).

The importance of BAX inhibitor-1 (BI-1) during cell death under various stresses was studied by Duan et al., 2010; Wang et al., 2012; Ramiro et al., 2016 & Lu et al., 2018. The expression of the BI-1 gene was up-regulated in the presence of 15% PEG, which is consistent with a previous report by Duan et al. (2010), who found that AtBI1 suppressed ER stress-induced PCD and assisted the cell in recovering from water stress damage in Arabidopsis. Wang et al. (2012) discovered that over-expression of TaBI-1 in wheat significantly reduces cell mortality in response to abiotic stress. Over-expression of the AtBI-1 increased sugarcane's water deficit tolerance capabilities, according to Ramiro et al. (2016). BI-1 levels in plants rose during leaf senescence (Duan et al., 2010), drought (Ramiro et al., 2016), and heat stress (Ramiro et al., 2016) (Lu et al., 2018). Over-expression of BI can thereby inhibit cell death activation (Duan et al., 2010). Senescence, as well as a variety of biotic and abiotic factors, influence the activation of programmed cell death (Duan et al., 2010). BI-1 gene expression is increased in stressful situations to prevent cell death and protect cells from the negative effects of stress (Watanabe and Lam, 2009). The BI-1 gene is over-expressed in plants under diverse biotic and abiotic stressors (Kawai et al., 1999; Yamada et al., 2001 & Watanabe & Lam, 2006).

In the current study, the expression of CESs was reduced in both treatments as compared to the control. This could be due to the fact that the expression of this gene has only been detected in response to biotic stress (Islam and Yun, 2016), where transcripts of the VfCXE gene showed active responses in grapevines against the pathogens E. ampelina, R. vitis, and B. cinerea. Under cadmium stress, Li et al. (2019) found that the esteras genes of the SaGELP gene family were expressed differently in different tissues and at different times in S. alfredii. Carboxylesterases catalyse the hydrolysis of carboxylic esters of biomolecules and control the synthesis and release of bioactive metabolites like hormones, which accumulate in plants as carboxylesters and amides (Gershater and Edwards, 2007). As a result, these enzymes are implicated in the systemic acquired immunity signaling pathway and are up-regulated in plants following pathogen infection (Chen et al., 2017). Carboxylesters also control cell death during HR, which is necessary to prevent plant disease (Marshall et al., 2003; Gershater and Edwards, 2007; Wheelock et al., 2008 & Chen et al., 2017). After 6 hours of cadmium exposure, the Esteras/lipase gene family was enhanced in Sedum alfredii (Li et al., 2019). During abiotic stress, CXE expression was elevated in the xylem of the vascular bundle of leaf tissue, and they were involved in defense-related signal
transduction cascades (An et al., 2008). Even though carboxylesterase activity has been identified in various investigations, the actual mechanism of CXEs in plants is unknown.

The function of PAL is to aid in the manufacture of various secondary metabolites in order to combat abiotic stress, as previously stated (Fossdal et al., 2007; Jeong et al., 2012; Kelij et al., 2013; Khakdana et al., 2018 & Liu et al., 2019). The PAL is vital for plant development, structural support, defence responses, and abiotic stimulant endurance, as well as for plant cell wall lignification. The current finding of a down-regulated PAL gene is consistent with Khakdana et al. (2018), who found that the expression of PALs in basil was either down- or up-regulated depending on the degree and duration of abiotic stress. In numerous tissues, the PAL gene family encodes several defensive chemicals such as flavanoids, phytoalexins, furanocoumarin, and cell wall components (Jahnen and Hahlbrock, 1988).

Plants' cellulose production is controlled by cellulose synthases (CSs) genes (Burton et al., 2006). Many scientists have investigated the mechanism of cell wall production during abiotic stress (Guerriero et al., 2014; Behr et al., 2015; Kestena et al., 2017 & Goncalves et al., 2019). The CSs gene was up-regulated in seedlings treated with 15% PEG and down-regulated in seedlings treated with 150 mM NaCl. This study supported by Li et al(2019)'s findings, which looked at the expression profiles of ZmCsl genes in maize during PEG-induced drought stress. After 60 hours of treatment, they discovered that more ZmCsl genes were up-regulated. PEG treatment increased the expression of four genes: ZmCslF6-1, ZmCslD1, ZmCslC2-1, and ZmCslC2-2. Under PEG-induced drought stress, Zhao et al. (2022) confirmed that the GhMCesA35 gene was involved in cellulose production in cotton. GhMCesA35 gene expression was up-regulated in Tm-1 and Hai-7124 during fibre secondary wall biosynthesis development. The findings revealed that this gene could boost the strength of the cell wall, which keeps the cell in shape and can withstand abiotic challenges like salt and dehydration. Under adverse conditions, it also modulates cell wall integrity (CWI) by inducing changes in lignin production and cellulose deposition to strengthen the cell wall (Delgado et al., 2003). The cell wall synthesis associated genes were dramatically up regulated in sweet orange grafted on the drought tolerant (‘Rangpur’) in drought condition (Goncalves et al., 2019), and the CesA6F gene was up regulated in salt situation in alfalfa plants (Behr et al., 2015), up regulation of MsCesA6-B gene after 96 h of heat and salt stress in alfalfa by Guerriero et al. (2014). Plants have a huge number of cellulose synthase genes, which are members of the glycosyltransferase family and are responsible for cellulose synthesis (Richmond, & Somerville, 2000 & Somerville, 2006).

Summary And Conclusions

Based on prior study and our findings, it can be inferred that teak seedlings demonstrated physiological and gene expressional changes under various abiotic stress situations. The formation of reactive oxygen species causes changes in chlorophyll content, membrane stability index, and relative water content. The cell, chloroplast membrane, and chlorophyll molecule are all effectively damaged by ROS. Induced ROS levels also disrupt cell membrane permeability, allowing cellular ions and materials to leak out. Under stress, the water content of the cell is also lowered due to lower water absorption to higher transpiration ratio. These factors will be useful in explaining the behavior of teak plant tissues in response to climate
change. As a result, teak seedlings that maintain these indices under stress are considered the most tolerant seedlings.

The genes MYB-3, HSP-1, BI-1, and CS-2 have major roles in protecting plant cells during stress conditions, according to the gene expression analysis of teak against abiotic treatment. MYB-3 regulates the expression of genes involved in cell division, scavenging enzyme production, hormone signaling, secondary metabolism, meristem development, and cellular morphogenesis. Heat shock proteins function as molecular chaperones, inhibiting the aggregation formation of other proteins, and participating in protein folding and mending misfolded conformers during prolonged stress or even in unstressed cells. Furthermore, a novel gene called BI-1 was discovered to have the highest expression under stress conditions, particularly in teak trees. As a result, this gene could be used to generate stress-tolerant teak genotypes through genetic engineering, as well as used as a flag marker for stress screening of teak germplasm. This gene has been found to play a role as a cell death suppressor, increasing during leaf senescence and in response to a variety of environmental stimuli such as salt, heat, and other factors, and its over-expression can block cell death activation. Furthermore, genetic engineering must be used to corroborate the functions of the genes under investigation. The study of tropical tree physiological and molecular mechanisms under drought is becoming increasingly important as climate change becomes more severe and wood demand rises.

Declarations

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Conflict of Interest: All authors have no conflict of interest.

Ethical approval: This article does not contain any studies with human participants performed by any of the authors.

References


52. Singh HS (2013) Tree wealth in the non-forest areas of Gujarat. Trees Outside Forest (TOF) - Third Tree Counting-2013. Gujarat Forest Department, Gandhinagar


**Figures**
Figure 1

See image above for figure legend.
Fig. 2: Chlorophyll content of teak seedlings under three treatments during experiment

Figure 2
See image above for figure legend.
Figure 3: Membrane stability index of teak seedlings under three treatments during experiment

See image above for figure legend.
**Figure 4**

See image above for figure legend.

**Supplementary Files**

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- Fig.1.1 Amplification and melt curve plot of Actin27.jpg
- Fig.1.2 Amplification and melt curve plot of MYB3.jpg
- Fig.1.3 Amplification and melt curve plot of HSP1.jpg
- Fig.1.4 Amplification and melt curve plot of BI1.jpg
- Fig.1.5 Amplification and melt curve plot of CES.jpg
- Fig.1.6 Amplification and melt curve plot of PAL1.jpg
- Fig.1.7 Amplification and melt curve plot of CS2.jpg
• SUPPLEMENTARYTABLE.docx