Principal Component Analysis of Changes due to Water Stress for Some Osmolytes, Pigments and Antioxidant Enzymes in *Gmelina arborea* Robx. Leaves from Trees Planted in Northern Colombia

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*Gmelina arborea* é uma espécie de alto impacto econômico devido a vantagens como árvore madeireira de rápido crescimento. A *G. arborea* é plantada na costa norte da Colômbia, especialmente nas planícies secas do Caribe. No entanto, condições de déficit hídrico a que estão periodicamente submetidos, gera perdas nas plantações. Neste estudo mediram se a atividade da catalase, peroxidase e ascorbato peroxidase, assim como o conteúdo de açúcares totais, açúcares redutores, proline, carotenóides, clorofila A, B em folhas coletadas em três estações climáticas diferentes. Um estudo de análises de componentes principais mostrou que o comportamento das variáveis medidas depende tanto da idade quanto do tempo de amostragem, além de uma correlação inversa entre as variáveis de clorofila A, B e total e as outras variáveis medidas.

*Gmelina arborea* is a tree having great economic impact due to its advantages as a fast-growing timber tree. *G. arborea* is currently being planted on the North Coast of Colombia, especially on the dry plains near the Caribbean. However, the stress conditions produced by drought to which they are periodically subjected lead to plantation loss. This study was thus aimed at measuring catalase, peroxidase and ascorbate peroxidase activities as well as total sugar, reducing sugar, proline, carotenoids, chlorophyll A, B and total chlorophyll content in *G. arborea* leaves sampled during three seasons of the year. Principal component analysis showed that the measured variables had patterns depending on age and season, in addition to an inverse correlation between chlorophyll A, B and total chlorophyll and the other measured variables.

Keywords: principal component analysis, water stress, oxidative stress, antioxidant system

Introduction

Plants are continuously exposed to unfavorable environmental conditions such as extreme temperatures and low water availability. Water deficit is most common as it results from reduced soil water availability and hot/dry weather conditions, causing continuous loss of water through transpiration and evaporation and thereby resulting in profound effects on plant growth, yield and productivity. Some physiological changes can be made to counteract such harmful effects arising from this event, such as increasing abscisic acid levels, stomatal closure, changes in cell osmolarity and increased enzymatic and non-enzymatic antioxidant concentration.1

Water deficit also results in important effects on photosynthesis due to minimizing carbon dioxide supply by the closure of stomata. However, when there is a gradual increase in deficit, acclimation may occur to minimize these effects due to osmotic adjustment, thereby enabling the chloroplasts to maintain volume by accumulating certain constituents in the stroma, as in the case of proline and some sugars.2,3 However, severe or long periods of low water availability can damage the photosynthetic machinery. Irradiance plays a vital role in changes taking place within a plant during water stress since the photosynthetic rate decreases and high irradiance produce excess energy resulting in the production of reactive oxygen species (ROS).1

ROS are molecules having high reactivity and occur during normal cell operation as part of the redox balance to activate and deactivate some protein activities.4 Its production arises from electron transfer to an oxygen molecule such as superoxide radical anion, hydrogen peroxide and hydroxyl radical, or spin alteration, as in
the case of singlet oxygen.\textsuperscript{3} ROS also act as an alarm for
imbalance in the plant, activating signaling cascades triggering short-term physiological responses or long-term
transcriptional ones.\textsuperscript{4} Furthermore, when ROS are produced in
large quantities they can be responsible for different
types of cell damage, such as lipid peroxidation, proteins
oxidation and even DNA damage.\textsuperscript{5-7}

Increased ROS levels are caused by several types of
environmental stress, creating what is known as oxidative
stress during which ROS produce an imbalance in the redox
state of the cell because they react with a large number of
structural molecules and so produce significant damage to
cell function.\textsuperscript{2} Two types of mechanism respond to such
altered redox states: a non-enzymatic system (consisting
of ascorbic acid, polyphenols, chalcones, tocopherols,
anthocyanins, carotenoids and glutathione) and enzyme
systems. Such enzymes can be classified into two broad
groups: (i) enzymes using $\text{H}_2\text{O}_2$ as substrate or cofactor
which are able to remove/neutralize $\text{H}_2\text{O}_2$ directly (i.e.
catalase (CAT),\textsuperscript{9} ascorbate peroxidase (APX),\textsuperscript{10} glutathione
peroxidase (GPOD),\textsuperscript{11} peroxyreductox (PRX),\textsuperscript{12} type 3
peroxidase (POD)\textsuperscript{13} and (ii) enzymes using different ROS
as substrate or cofactor (i.e. superoxide dismutat (SOD)\textsuperscript{14}
and glutathione reductase (GR)).

Catalase (CAT) (EC. 1.11.1.6) is located in peroxisomes
and is responsible for controlling the flow of $\text{H}_2\text{O}_2$ by
catalyzing its disproportion to water and oxygen. It
is usually a tetramer wherein each subunit contains a
heme group.\textsuperscript{15} Peroxidases (POD) (EC. 1.11.1.7) are
oxydoreductase metalloenzyme series reducing organic
and inorganic peroxides into their corresponding alcohols
by using a cysteine active site.\textsuperscript{16} They are located in the
cytosol in soluble form and are bound through ionic
or covalent bonds on the cell wall.\textsuperscript{17} Ascorbate peroxidase
(APX) (EC. 1.11.1.11) is one of the most important enzymes
in hydrogen peroxide and nitrogen detoxification in
chloroplasts; it participates in the glutathione-ascorbate cycle.
This enzyme uses $\text{H}_2\text{O}_2$ as substrate together with ascorbate
anion to generate monodehydroascorbate (MDA) and
dehydroascorbate (DHA).\textsuperscript{18,19}

\textit{G. arborea} is a native South-eastern Asia specie and has
been introduced into South America. It belongs to the family
Verbenaceae and is characterized by rapid growth and
it reaches up to 30 m in height and 100 cm in diameter.
This species is recognized for its potential in ecosystem
recovery and that of the environment.\textsuperscript{20,21} \textit{G. arborea} has
been planted on the Northern Coast of Colombia, especially
on the dry plains near the Caribbean. However, the water
deficit conditions to which they are periodically subjected
cause plantation loss, increased costs due to re-seeding and
the need for greater use of irrigation water.

This study was aimed at determining total sugar,
reducing sugars, proline, carotenoids, chlorophyll A, B
and total chlorophyll and total protein content as well as
catalase, peroxidase and ascorbate peroxidase activities
during three climatic seasons in the leaves of \textit{G. arborea}
Robx trees planted in northern Colombia.

\section*{Experimental}

\subsection*{Apparatus}

A SmartSpec Plus spectrophotometer (Bio-Rad) was
used for taking spectrophotometric measurements for
determining chlorophyll A, B, total chlorophyll, carotenoids
and proline content and determining catalase, peroxidase
and ascorbate peroxidase activities. An ImarkMicroplate
Absorbance Reader was used for taking total and reducing
sugars and total protein spectrophotometric measurements.

\subsection*{Reagents and solutions}

All chemicals were analytical-reagent grade or better.
All solutions and dilutions were prepared with ultrapure
water (Thermo Scientific Barnstead RO, Reverse Osmosis
System).

\subsection*{Samples}

The leaf tissue samples were taken from \textit{G. arborea}
Robx clone 79 from Pizano SA Monterrey Forestal Ltda
plantation in Zambrano (Bolivar), northern Colombia; they
were collected during the three study periods: rainy season
(September-October), transition to dry season (December)
and dry season (February-April). Three-hour sampling
(8, 10 and 12 h) and three ages (seedling, juvenile and
adult) were used. The seedlings were sown in the field
on August 6\textsuperscript{th} 2008, the samplings (juvenile trees) on
October 10\textsuperscript{th} 2007 and adult trees on May 25\textsuperscript{th} 2004.

The leaves were collected from the mid-section of
three trees per age and stored at $-10$ °C; they were then
macerated with liquid N\textsubscript{2} to a fine powder which was kept
at $-10$ °C until final processing. Climatic data: maximum
temperature and rainfall were obtained from the IDEAM
weather station in Monterrey Forestall (Figure S1).

\subsection*{Analytical determination}

Material was macerated in triplicate using the
following determination: catalase (CAT) activity
(\textmu g mol $\text{H}_2\text{O}_2$ min$^{-1}$ mg$^{-1}$ protein) according to Aebi,\textsuperscript{22}
peroxidase (POD) activity ($\Delta A_{436nm}$ min$^{-1}$ mg$^{-1}$ protein)
according to Kireyko et al., ascrobate peroxidase (APX) activity (nmol oxidized ascorbate min⁻¹ mg⁻¹ protein) according to Nakano and Asada, protein content according to Bradford (mg g⁻¹ plant material), carotenoids and chlorophylls A, B and total chlorophyll (mg g⁻¹ plant material) according to Lichtenthaler, total sugar content (mg g⁻¹ plant material) according to DuBois et al., reducing sugar content (mg g⁻¹ plant material) according to Nelson and Somogyi, and proline content (µg g⁻¹ plant material) according to Bates et al.

All methods were standardized in the Plant Physiology and Biochemistry Research Laboratory of the Universidad Nacional de Colombia and details can be found in the Plant Physiology Experiments manual.

Data analysis

A data matrix containing 27 rows (samples) and 13 columns (variables) was built. The pattern recognition technique used in this work was principal component analysis (PCA): this is a procedure that allows exploring the data structure, the relationship between objects, the relationship between objects and variables and overall correlation of the variables. MATLAB 7.9.0529 (R2009b) for Windows (MathWorks) was used for PCA.

Results

Table S1 shows the results for determining total and reducing sugars, proline, chlorophyll A, B, total chlorophyll, carotenoids, total protein and catalase, peroxidase and ascorbate peroxidase activity.

Total sugar, reducing sugar, proline, carotenoids, chlorophyll content and CAT, POD and APX activity were used as descriptors for principal component analysis; this analysis was carried out with auto-scaling data.

It was found that the first component accounted for 64.21% of variance and the second one to 14.11% of variance. The loading plot (Figure 1) showed that principal component PC1 gave chlorophyll A, chlorophyll B and total chlorophyll as dominant variable, appearing to be negative PC1 values. Proline, catalase, carotenoids, season, peroxidase and reducing sugars appeared to be positive values for PC1, so there was an inverse correlation between chlorophyll A, B and total chlorophyll compared to the other variables. Dominant variables for the second component (PC2) were chlorophylls A and B, total chlorophyll and total protein; they displayed positive PC2 values. It is worth noting that time was not a relevant variable for either component (PC1 and PC2), however, time appeared to have negative values for the third component (not shown) which explained 7.73% of variance, having an inverse correlation with protein.

The score plot (Figure 2) clearly shows the presence of three different groups on the PC1 axis corresponding to sampling time. PC2 shows three different age groups. These results showed that the measured variables had a pattern dependent on both season and age.

Simultaneous analysis of the two charts showed that adult specimens had a direct correlation with sugar content; seedlings were correlated with protein, carotenoids, chlorophyll A, B and total chlorophyll content; while juveniles occupied an intermediate position.

Discussion

Water stress leads to reduced cell water content, in turn leading to loss of lipid bilayer integrity ending in loss of function, selectivity, disruption and affects the activity of
some enzymes. Moreover, in the event of stress-related
drought, there are three types of signals or response which
are designed to compensate for water loss. First, conditions
are maintained which produce efficient osmotic water use
(ABA production) and maintain homeostasis under stress
by an increase in molecules consistent with sugar, polyol,
proline cell structures. Secondly, ROS are controlled by
enzymatic and molecular systems and, thirdly, cell division
is coordinated to meet the requirements of plants under
stress.\textsuperscript{33}

The present study provided evidence that \textit{G. arborea}
increased sugar and proline content during the dry season
compared to rainy periods and transition to dry season
(Table S1) as an osmoregulatory response to prevent cell
dehydration. This behavior has been reported in other studies
of plants subjected to water stress in controlled conditions\textsuperscript{34,35}
or in field conditions\textsuperscript{46} (as our case study). This response
occurs because plants tend to compensate for cellular drought
stress through osmoregulatory production of proline, glutamate, glycine betaine, carnitine, mannitol, sorbitol,
fructans, polyols, trehalose, sucrose, some hexoses and
oligosaccharides. These molecules help maintain a hydrated
state and promote resistance to dehydration by maintaining
leaf turgor.\textsuperscript{37,38} It is assumed that sugars also play a role in
stabilizing some macromolecules and cellular structures\textsuperscript{49}
are involved in controlling ROS during water stress\textsuperscript{40} and
assist in maintaining certain physiological functions,
such as partial stomatal opening, photosynthesis and cell
growth.\textsuperscript{41} Moreover, it has been proposed that proline
affects some effectors involved in the expression of genes
related to plant tolerance to different types of stress.\textsuperscript{2,42}
It also has a stabilizing effect on protein and membrane
structure, is an inducer of osmotic stress-related genes\textsuperscript{43}
and is a source of readily-available carbon and nitrogen in cell
re-hydration.\textsuperscript{44} This study (like many others) has shown that
there is an increase in total soluble sugars and proline during
water stress conditions\textsuperscript{41,42,45,46} as a strategy for preventing
drying out and damage from oxidative stress.

Increased carotenoid content is another response to
energy dissipation level and carbon and nitrogen content
regulation to increase the excess energy of the receptor
molecules and use chlorophyll as a source of carbon and
nitrogen.\textsuperscript{47} There is perfect coordination between light-
induced electron transfer in thylakoid membrane and
carbon dioxide fixation by the Calvin cycle in the stroma
in natural conditions; however, stress results in an
imbalance which triggers ROS formation that damages the
photosynthetic apparatus.\textsuperscript{48}

The \textit{G. arborea} study reported here found decreased
chlorophyll A, B and total chlorophyll content and an
increase in carotenoids during the dry season compared
to the rainy and transition to dry season. Pigment study
is important from an ecophysiological viewpoint as
it provides information about productivity, stress and
degradative events and limiting nutrients. Alterations in
photosynthetic pigment composition may be related to
photoacclimation.\textsuperscript{49} High irradiance acclimated cells may
contain high concentrations of carotenoids in relation to
chlorophyll A. Yellow-orange carotenoids are associated
with membrane-embedded proteins within chloroplasts
where they interact with acceptor molecules and carriers.
They have a protection-inducing photooxidative role against
damage to the photosynthetic apparatus, dissipating excess
light absorbed by pigment antenna.\textsuperscript{50,51} Similar results
have been reported for other plants subjected to water stress.
Nayyar and Gupta\textsuperscript{52} found a significant decrease in
chlorophyll content in \textit{Triticum aestivum} and \textit{Zea mays}
plants subjected to water stress, and Jung\textsuperscript{53} found a decrease
in chlorophyll content in mature \textit{Arabidopsis thaliana}
leaves subjected to water stress; however, no changes were
found in carotenoids content compared to control.

The results of CAT, POD and APX activities in
\textit{G. arborea} showed significant increase during the dry season
compared to the rainy season and transition to dry season.
Other studies have reported similar behaviors: Bai \textit{et al.}\textsuperscript{34}
found increased SOD activity during severe water stress
management in maize plants (\textit{Zea mays} L.) and increased POD
activity when subjected to moderate stress and severe stress.
Ozkur \textit{et al.}\textsuperscript{55} noted significant increases in SOD, POD and
CAT activities in leaves from caper (\textit{Capparis ovate}
Def.) plants subjected to water stress induced by adding
polyethylene glycol (PEG). Nayyar and Gupta\textsuperscript{52} studied the
effect of low, moderate and severe water stress on C3
(\textit{Triticum aestivum}) and C4 plants (\textit{Zea mays}), finding that
leaves’ CAT and APX activity was greater for moderate
stress in both types of plant.

Conclusions

Principal component analysis clearly established the
presence of three groups corresponding to season (rainy
season, transition to dry season and dry season) and
three age-related groups (seedling, juvenile and adult).
Increased osmoregulatory compound content and activity
of the enzymes being studied (forming part of antioxidant
enzymes) indicated that \textit{G. arborea} exhibited oxidative
stress due to water stress during the dry season. It showed
that the plant responded to such oxidative stress by
increasing the activity of particular enzymes catalyzing
the decomposition of chemical ROS species which are
dangerous for cells and synthesizing compounds stabilizing
cell structures and preventing desiccation.
Supplementary Information

Supplementary data (Table S1 and data concerning climate Figure S1) are available free of charge at http://jbcs.sbq.org.br as pdf file.

Acknowledgments

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References


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**Climate**

Figure S1 shows the maximum temperature ($T_{max}$) and rainfall pattern during the study period. Rainfall during September and October had similar, high values. The values recorded during November were because of La Niña. Rainfall became minimal during December and the dry season began in January.

![Graph showing temperature and precipitation](image)

**Figure S1.** Average daily maximum temperature ($T_{max}$) and rainfall from September 2008 to April 2009.

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Table S1. Results for determining total (AZT) and reducing (AZR) sugars, total protein (PRT), chlorophyll A (ChlA), B (ChlB), total (ChlT) chlorophyll and carotenoids (CRT) in mg g⁻¹ plant material, proline (PRL) in µg g⁻¹ plant material, and enzyme activity for catalase CAT (µmol H₂O₂ min⁻¹ mg⁻¹ protein), peroxidase POD (ΔA_436nm min⁻¹ mg⁻¹ protein) and ascorbate peroxidase APX (nmol oxidized ascorbate min⁻¹ mg⁻¹ protein) in three determinations ± S.D.

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<th>AZR</th>
<th>PRL</th>
<th>ChlT</th>
<th>ChlA</th>
<th>ChlB</th>
<th>CRT</th>
<th>PRT</th>
<th>CAT</th>
<th>POD</th>
<th>APX</th>
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*Average of three determinations ± S.D.*