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Does leaf turgor loss point (π_{tlp}) differ between nitrogen fixers and non-nitrogen fixers? - A short research

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Abstract

Turgor pressure is a critical factor in maintaining plant cell structure and function, influencing growth and resistance to environmental stress. Nitrogen fixation in some plant species may contribute to differences in water regulation and turgor pressure points. This study aims to compare the leaf turgor loss points (π_{tlp}) of nitrogen-fixing plants with those of non-nitrogen-fixing plants under both normal and water-limited conditions. Measurements were taken using a psychrometer across different time points to assess variation in turgor pressure dynamics. Results indicated that nitrogen-fixing plants generally maintained higher leaf turgor pressure than non-nitrogen-fixing plants, particularly under water stress conditions. These findings suggest that nitrogen fixation may confer advantages in water management, improving drought resilience in nitrogen-fixing species. Further research could explore the underlying physiological mechanisms and their implications for crop improvement.

Keywords: *Leaf turgor loss point; nitrogen-fixers; non-nitrogen fixers; drought tolerance; plant stress.*

Introduction

Nitrogen is a requisite element for plant growth and development as it is responsible for the production of amino acids, which are the building blocks of protein. Additionally, it is an essential component in the synthesis of nucleic acid (DNA and RNA), essential for all living organisms (1). Research studies have shown that the efficient utilization of nitrogen by plants is responsible for an increased root biomass in plants along with a widespread network of roots for better water absorption (2). Although nitrogen makes up 78% of the atmosphere and 98% of the soil as organic nitrogen, plants cannot directly utilize it. Instead, nitrogen must be fixed either through fertilizer production or by microorganisms that form symbiotic relationships with plants (3). This study examines two plant groups—nitrogen fixers and non-nitrogen fixers—to investigate how nitrogen fixation influences turgor loss point (TLP) in a plant. The findings aim to reveal whether nitrogen fixation contributes to better plant survival under drought conditions. The nitrogen-fixing group of plants include *Caesalpinia pulcherrima*, a common ornamental and medicinal plant in India, belonging to the Caesalpiniaceae family (4,5). *Pongamia pinnata*, a nitrogen-fixing tree from the Fabaceae family https://winrock.org/pongamia-pinnata-a-nitrogen-fixing-tree-for-oilseed (6). *Albizia saman,* belonging to the Leguminosae family, forms nitrogen fixing symbiosis with many strains of Rhizobium, and readily fixes nitrogen by forming root nodules (7). *Albizia lebbeck*, another species of genus *Albizia* which possesses nitrogen fixing properties (8). Lastly, *Saraca asoca*, a nitrogen fixer that belongs to family Fabaceae and has long existed as a part of Indian traditional medicine, specifically to treat gynecological disorders (9). The non-nitrogen fixing group of plants include *Terminalia arjuna,* commonly known as arjuna tree, which belongs to the Combretaceae family (10). *Terminalia bellirica*, another plant species of the same family (11). *Butea monosperma,* a Fabaceae family member (12). *Wrightia tinctoria,* a non-nitrogen fixer, which belongs to the family Apocynaceae (13). The last non-nitrogen fixer of this group is *Azadirachta indica* commonly known as neem which occupies a significant position in Indian traditional medicine (14). The trait of leaf turgor loss point (π_{tlp}) discussed in this study reflects a plant's capacity to maintain turgor pressure during leaf dehydration, and is an important predictor of its response to drought (15). Traditionally, turgor loss point (TLP) was measured using an approach of pressure-volume curve also known as pressure-bomb technique developed by Scholander and colleagues (16). It deals with theoretical analysis of equilibrium water relations of a twig's cells taking into consideration the fact that each cell has unique shape, solute concentration, fluid content and mechanical strength given by its cell wall structure and attachment to neighboring cells (17). Pressure-volume curves summarise leaf level responses to increasing water scarcity (18). Recent studies have demonstrated that the measurement of TLP can be effectively achieved using vapor pressure osmometers and psychrometers (19,21). Studies suggest that plants with more negative TLP can better resist the dehydration of leaves, which in turn helps them to sustain physiological processes like stomatal conductance, photosynthesis and growth even under scarcity of water (22-28). This research study mainly relies on the leaf TLP estimation of nitrogen fixers and non-nitrogen fixers for the estimation of better drought tolerance. This study quantified differences in leaf turgor loss points (π_{tlp}) between two plant groups i.e., nitrogen fixers and non-nitrogen fixers to assess which plant group has a better tolerance under drought conditions.

Materials and Methods

Sample Collection: The plants of both the groups (nitrogen fixers and non-nitrogen fixers) were collected from the National

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Centre for Biological Sciences (NCBS), Bengaluru, and the Gandhi Krishi Vigyana Kendra (GKVK), Bengaluru, which are specifically planted for research purposes.

Sample preparation: The leaf samples for the estimation of TLP were collected one day before and stored in a beaker with proper labeling indicating the date of collection, name of the person who collected the sample, plant species name, and replica number (abbreviated as 'R'). Five different replicates (R_1, R_2, R_3, R_4 and R_5) for each plant species were sampled. The leaf samples were cut from the plants by snipping the leaf sheath and making a base cut underwater, taken in a beaker, and were kept submerged to avoid cavitation. Then, the beakers with the leaf samples were kept inside a zip lock bag with moist tissue to ensure that the air inside the bag remained humid. These samples were stored in a dark place and were allowed to rehydrate for about 20-24 hours and were only taken out during TLP estimation.

Materials Required: Psychrometer, tissue roll/ paper towels, aluminum foil, cork borer or puncher, sharp-tipped tweezers/insect pin, liquid nitrogen, protective gloves, forceps/ tongs, pipette tip of any size, leaf samples (rehydrated for 20-24 hours).

Osmotic Pressure (π_{osm}) estimation using Psychrometer: On the day of measurement the psychrometer was allowed to equilibrate for 20-30 minutes after applying grease on the outer corners of the measurement chamber. The chamber was carefully closed and the lid was tightly secured with the help of masking tape. After equilibration the prepared leaf samples were taken out and gently wiped using tissue paper, to ensure no water content was present on the leaf surface at the time of measurement. After patting dry the leaf, it was gently rubbed with sandpaper to remove any trichomes present on the surface. Then the leaf was quickly punched using a paper puncture and was covered with aluminum foil within 30 seconds. The covered leaf disc was frozen in liquid nitrogen for about 2 minutes. After 2 minutes, the leaf disc was taken out, carefully opened and then poked 15-20 times with the help of an insect pin. The leaf disc was then carefully placed in the measuring chamber and the chamber was sealed again using masking tape. The first reading was taken after 2 minutes in the ICT software and the rest of the readings were taken with an interval of 10 minutes until stabilized readings were observed. After taking the readings the measuring chamber was carefully cleaned using distilled water and tissue paper and dried using nitrogen gas. The same procedure was repeated to find the osmotic potential of each leaf sample.

Calculation of leaf turgor loss point (π_{tlp}) using osmotic potential (π_{osm}): A research study conducted by Megan K. Bartlett on "Rapid determination of comparative drought tolerance traits: using an osmometer to predict turgor loss point" provided us with one of the first regression equations that allows the prediction of turgor loss point (π_{tlp}) from osmotic potential (π_{osm}). The equation stated as follows; $\pi_{tlp} = 0.832\pi_{osm} - 0.0631$ (21).

The TLP of all the plant species were calculated using the above formula.

Results and Discussion

The result of the study showed a significant difference in turgor loss point values between nitrogen fixers and non-nitrogen fixers. The mean TLP values (Table 2 and Figure 2) of nitrogen fixers were observed to be -2.55848 MPa and non-nitrogen fixers were observed to be -1.842724 MPa. Both the values show significant differences. Research studies have proven that the plants with more negative TLP value have better survival rate during conditions of water stress. It is because of the reason that even under low water availability these plants can support metabolic functions like stomatal closure, photosynthesis and growth (22-28) . As observed in the results, nitrogen fixers have more negative TLP value than non-nitrogen fixers, suggesting their potential for better survivability under drought conditions.

Table 1: TLP values of all the replicates of both the plant groups, i.e., nitrogen fixers and non-nitrogen fixers

Plant groups	Plant species	Species code	TLP value (MPa)				
			R-1	R-2	R-3	R-4	R-5
Nitrogen fixers	*Caesalpinia pulcherrima*	CAEPUL	-2.23676	-2.26172	-2.6172	-2.20348	-2.22012
	Pongamia pinnata	PONPIN	-2.69436	-2.72764	-2.68604	-2.69436	-2.61948
	Albizia saman	ALBSAM	-2.62780	-2.70268	-2.62780	-2.61116	-2.56124
	Albizia lebbeck	ALBLEB	-2.76092	-2.86908	-2.91900	-2.73596	-2.70268
	Saraca asoca	SARASO	-2.44476	-2.39484	-2.45308	-2.43644	-2.49468
Non-nitrogen fixers	*Terminalia bellirica*	TERBEL	-1.67932	-1.65436	-1.62108	-1.67932	-1.63772
	Terminalia arjuna	TERARJ	-1.85404	-1.86236	-1.84572	-1.82908	-1.82076
	Butea monosperma	BUTMON	-2.01212	-1.97052	-2.00380	-2.03708	-2.10364
	Wrightia tinctoria	WRITIN	-1.68764	-1.63772	-1.69596	-1.70428	-1.64604
	Azadirachta indica	AZAIND	-2.10364	-1.97884	-1.98716	-2.06204	-2.03708

Table 2: Compiled data showing the difference in Average Turgor Loss Point (π_{tlp}) between plant Groups

Plant Group	Species Name	Species Code	Average π_{tlp} (MPa)	Average π_{tlp} (MPa) for each Plant group
Nitrogen Fixers	*Caesalpinia pulcherrima*	CAEPUL	-2.236780	-2.55848
	Pongamia pinnata	PONPIN	-2.684376	
	Albizia saman	ALBSAM	-2.626136	
	Albizia lebbeck	ALBLEB	-2.797528	
	Saraca asoca	SARASO	-2.444560	
Non-nitrogen fixers	*Terminalia bellirica*	TERBEL	-1.654360	-1.842724
	Terminalia arjuna	TERARJ	-1.842398	
	Butea monosperma	BUTMON	-2.008792	
	Wrightia tinctoria	WRITIN	-1.674328	
	Azadirachta indica	AZAIND	-2.033752	

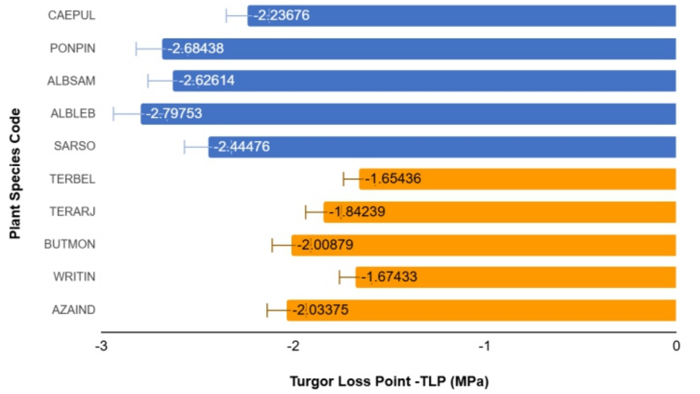

Figure 1- The average turgor loss point (π_{tlp}) with standard error shown in nitrogen fixers and non nitrogen fixers

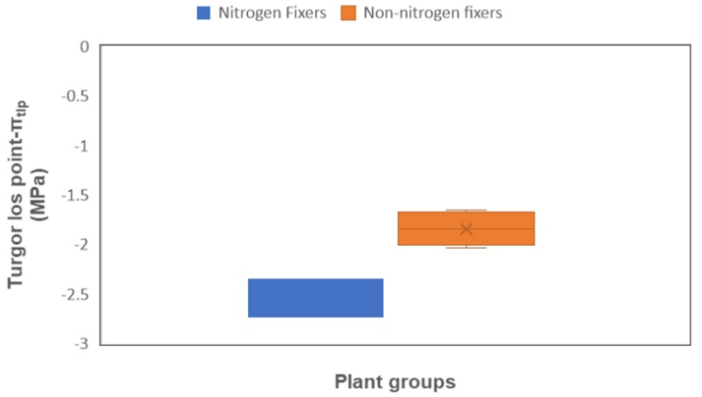

Figure 2- Box and whisker plot showing the grouped comparison of turgor loss point (π_{tlp}) of nitrogen fixers and non-nitrogen fixers

Conclusion

The comparative study between the nitrogen fixers and non-nitrogen fixers taking into consideration their turgor loss point aimed to investigate how this trait is associated with a plant's better survivability under water stress conditions. Through TLP measurement and comparison the study revealed a significant difference in the TLP values between the two plant groups, with nitrogen fixers exhibiting more negative TLP value suggesting their better adaptability under water stress conditions. The studies going on in the field of drought tolerance mechanisms in plants can have significant contributions regarding sustainable agricultural practices. Additionally, exploring the ecological consequences of nitrogen fixation on plant community dynamics and ecosystem functioning could provide valuable insights into broader implications of this study.

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