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THE UNIVERSITY
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AUSTRALIA

**IDENTIFICATION OF TRANSGENIC
PLANT SPECIES USING NON-INVASIVE
THz SPECTROSCOPY FOR ADVANCING
NATURAL AND ORGANIC FARMING
PRACTICES**

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THE INDIAN INSTITUTE OF TECHNOLOGY DELHI

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THE UNIVERSITY OF QUEENSLAND

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Identification of Transgenic Plant Species using Non-Invasive THz Spectroscopy for Advancing Natural and Organic Farming Practices

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MS RNA Sciences, B.Tech Genetic Engineering



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&

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Supervisor Certification

This is to certify that the thesis entitled “**Identification of Transgenic Plant Species using Non-Invasive THz Spectroscopy for Advancing Natural and Organic Farming Practices**” is being submitted by **Ms. Urbi Kundu** to the Indian Institute of Technology Delhi and The University of Queensland for the award of the degree of **Doctor of Philosophy** is a record of *bonafide* research work carried out by her. **Ms. Urbi Kundu** has worked under our guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to our knowledge has reached the requisite standard. The results contained in this thesis are original and have not been submitted, in part or full, to any other University or Institute for the award of any other degree or diploma.



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Abstract

With growing concerns over food security and plant genetic diversity conservation, we aimed to develop a non-invasive, rapid, robust, and high-fidelity technique to discriminate between genetic variants. Our study focused on Terahertz (THz) spectroscopy and imaging to distinguish between genetic variants of the *Allium* genus rapidly and accurately. This was done by measuring the cellular water dynamics in the epidermal tissues as it interacts with the biomechanics and the biomolecules within the cells over a period of time through THz time domain spectroscopy, continuous wave THz frequency domain spectroscopy and by using laser feedback interferometry with THz quantum cascade lasers. The evaporation profiles of the samples were then processed to determine that the biomechanical breakdown was frequency independent and unique to each type of genetic variant based on their biochemistry and biomechanics. We were able to show that the water dynamics, when considered in the form of the temporal evolution of the trajectory of the plant's response to terahertz radiation probe, and measured by a coherent terahertz detector, provides unique signature of the genetic makeup of the plant. Therefore by exploring these trajectories, we discriminate between closely related variants of the same genus. The effectiveness of THz spectroscopy and imaging allowed us to capture these time lapse changes in high resolution, leading for us to deem this technique to be suitable for real-time observation of minute changes in cellular water dynamics, and in the future, could be used for swift and efficient genetic discrimination in agricultural and genome conservation applications.

सार

बढ़ती खाद्य सुरक्षा चिंताओं और पौधों की आनुवंशिक विविधता संरक्षण देखते हुए, हमने आनुवंशिक रूपों के बीच विभेद करने के लिए एक गैर-आक्रामक, त्वरित, मजबूत और उच्चतद्रूप तकनीक विकसित करने का लक्ष्य रखा। हमारा अध्ययन टेराहर्ट्ज़ स्पेक्ट्रोस्कोपी और चित्र पर ध्यान केंद्रित है ताकि “एलियम” जीनस के आनुवंशिक रूपों के बीच तेजी और अचूक तरीके से अंतर किया जा सके। ये काम एपिडर्मल ऊतकों कोशिकीय तरल गतिकी को मापकर और कोशिकीय जैवयांत्रिकी पर उसका परस्पर प्रभाव को एक लम्बे समय तक अध्ययन करके किया गया है। इसके लिए क्षेत्र स्पेक्ट्रोस्कोपी, टेराहर्ट्ज़ निरंतर तरंग आवृत्ति क्षेत्र स्पेक्ट्रोस्कोपी का उपयोग किया गया है। इसके अलावा टेराहर्ट्ज़ क्वांटम कैस्केड लेज़रों के साथ लेजर फीडबैक इंटरफेरोमेट्री का उपयोग करके किया गया था। नमूनों के वाष्पीकरण प्रोफाइल को तब तक संसाधित किया गया था ताकि यह निर्धारित किया जा सके कि बायोमेकेनिकल टूटना आवृत्ति स्वतंत्र था और प्रत्येक प्रकार के आनुवंशिक रूप के लिए उनके जैव रसायन और बायोमेकेनिक्स के आधार पर विशिष्ट था। यह टेराहर्ट्ज़ समय क्षेत्र स्पेक्ट्रोस्कोपी, टेराहर्ट्ज़ निरंतर तरंग आवृत्ति क्षेत्र स्पेक्ट्रोस्कोपी के माध्यम से टेराहर्ट्ज़ समय के बाद जीवीय कोशिका एपिडर्मल ऊतकों में जीवीय कोशिका जल गतिविधियों को मापकर किया गया था और टेराहर्ट्ज़ क्वांटम कैस्केड लेज़रों के साथ लेजर फीडबैक इंटरफेरोमेट्री का उपयोग करके किया गया था। नमूनों के वाष्पीकरण प्रोफाइल को संसाधित किया गया था ताकि यह निर्धारित किया जा सके कि बायोमेकेनिकल टूटना आवृत्ति स्वतंत्र था और प्रत्येक प्रकार के आनुवंशिक रूप के लिए उनके जैव रसायन और बायोमेकेनिक्स के आधार पर विशिष्ट था। हम यह दिखाने में सक्षम थे कि पानी की गतिशीलता, जब टेराहर्ट्ज़ विकिरण जांच के लिए पौधे की प्रतिक्रिया के प्रक्षेपवक्र के अस्थायी विकास के रूप में विचार किया जाता है, और एक सुसंगत टेराहर्ट्ज़ डिटेक्टर द्वारा मापा जाता है, तो पौधे की आनुवंशिक संरचना का अद्वितीय हस्ताक्षर प्रदान करता है। इसलिए इन प्रक्षेप पथों की खोज करके, हम एक ही जीनस के निकट संबंधी वेरिएंट के बीच विभेद करते हैं। THz स्पेक्ट्रोस्कोपी और इमेजिंग की प्रभावशीलता ने हमें उच्च रिज़ॉल्यूशन में समय के साथ होने वाले परिवर्तनों को देख सके जिससे हमने इस तकनीक को सेलुलर जल गतिशीलता में छोटे छोटे परिवर्तनों को वास्तविक समय अवलोकन के लिए उपयुक्त माना, और भविष्य में इसका उपयोग कर सकते हैं। कृषि और जीनोम संरक्षण अनुप्रयोगों में तीव्र और कुशल आनुवंशिक विभेद के लिए।

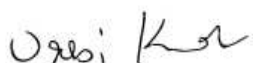
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Publications included in this thesis

1. [1] **Urbi Kundu**, Aparajita Bandyopadhyay, and Amartya Sengupta, Novel diagnostic scheme biomechanical discrimination of cells using THz-TD transmission spectroscopy, *European Conferences on Biomedical Optics (ECBO), OSA Technical Digest* 2021
2. [2] **Urbi Kundu**, Aparajita Bandyopadhyay, Karl Bertling, Aleksandar D. Rakić and Amartya Sengupta, Rapid genetic discrimination in plants through terahertz spectroscopy, *Proceedings, Terahertz Photonics II*; , Vol. 12134, p. 121340F, SPIE, 2022
3. [3] **Urbi Kundu**, Jari Torniainen, and Aleksandar D. Rakić, Variability in plant cell water dynamics as a genetic discriminatory method using terahertz quantum cascade laser based laser feedback interferometry, *Proceedings, Photonic Technologies in Plant and Agricultural Science*; , Vol. 12879, p. 128790K, SPIE, 2024
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1. [6] Mayuri Kashyap, Jari Torniainen, Karl Bertling **Urbi Kundu** and Aleksandar D. Rakić, Coherent terahertz laser feedback interferometry for hydration sensing in leaves, *Optics Express*; , Vol. 31, p. 23877, Optica Publishing Group, 2023

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1. Conceptualization and design of the project: Karl Bertling, Aparajita Bandyopadhyay, Aleksandar D. Rakić, Amartya Sengupta
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To Dr. M. S. Swaminathan, who put me on this path.

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List of Abbreviations and Symbols

Abbreviations

THz	Terahertz
FTIR	Fourier transform infrared
QCL	Quantum cascade laser
LFI	Laser feedback interferometry
IR	Infrared
VCA	Voice coil actuator
MQWHs	multiple quantum well heterostructures
Tx	Transmitter
R/Rx	Receiver
E	Emitter
PCA	Photoconductive antenna
DC	Direct current
AC	Alternate current
OAPMs	Off axis parabolic mirrors
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
PCR	Polymerase chain reaction
ELISA	Enzyme-linked immunosorbent assay
DIC	Differential Interference Contrast
LT	Low temperature
RD-SOS	Radiation-damaged silicon on sapphire
PCA	Principal component analysis
DA	Discriminate analysis
DLS	Partial least squares
GM(O)	Genetically modified organism
WT	Wild type
CTAB	Cetyltrimethylammonium bromide
EDTA	Ethylenediaminetetraacetic acid
HCl	Hydrochloric acid

ATR Attenuated total reflectance

Symbols

μm	micrometer
nm	nanometer
fs	femtosecond
ps	picosecond
dB	Decibels
K	Kelvin
kV	Kilo volts
mm	millimeter
GHz	Gigahertz
MHz	Megahertz
mW	milliwatts
μW	microwatts
M pixel	Mega pixel
A	Absorbance
$\hat{\phi}$	Normalized phase
rpm	Revolutions per minute
M	Molar
$^{\circ}\text{C}$	Celsius
g	grams
μl	microliter
%	percentage
kb	kilo basepairs
ng	nanogram
