ASIAN HERBS IN SPACE(AHiS)

Dr. Khairun Hisam Nasir
&
AHiS Malaysia Members Committee

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National planetarium, Kuala Lumpur

SLIDE

- INTRODUCTION
- STATUS ON GROUND EXPERIMENT
  - Germination of Holy Basil (*Oscimum Sanctum*) Seed
  - RNA Extraction
  - Prelimenary Microscopic and Macroscopic studies
  - Metabolomic Analysis
- CONCLUSION

INTRODUCTION

- The Asian Herbs in Space (AHiS) programme activities provided by Asian Beneficial Collaboration for Kibo Utilization (Kibo-ABC) under provision of Japan Aerospace Exploration Agency (JAXA),
- Kibo is nick name for Japanese Experiment Module (JE) for the International Space Station (ISS) developed by JAXA
- Collaboration provides scientist with opportunities experiments under space conditions including microgravity.

INTRODUCTION (cont)

- Through AHiS, a batch of Malaysia herb candidate, Basil (*Ocimum Santcum)* will be grown for 25 – 30 days onboard of the ISS, tentatively on May to September 2016
- Astronauts will assist to activate the germination, monitor and observe the germination in space and transmit the image back to earth. The herbs growth during the mission will be also collected and brought back for scientific sample analysis

INTRODUCTION (cont)

- Holy basil (*Ocimum sanctum, 2n=16)* , draft nuclear genome sequence of 386 Mb
- family-Lamiaceae
- cultivated throughout Southeast Asian tropics
- contains high in vitamin A and C
- Pre-clinal study on animal showed antidiabetic effect (Narendhirakkannan et al, 2006), wound healing activity (Udupa et al, 2006), antiinflammatory (Kelm et al 2000) and anticancer properties (Karthikeyan et al, 1999).

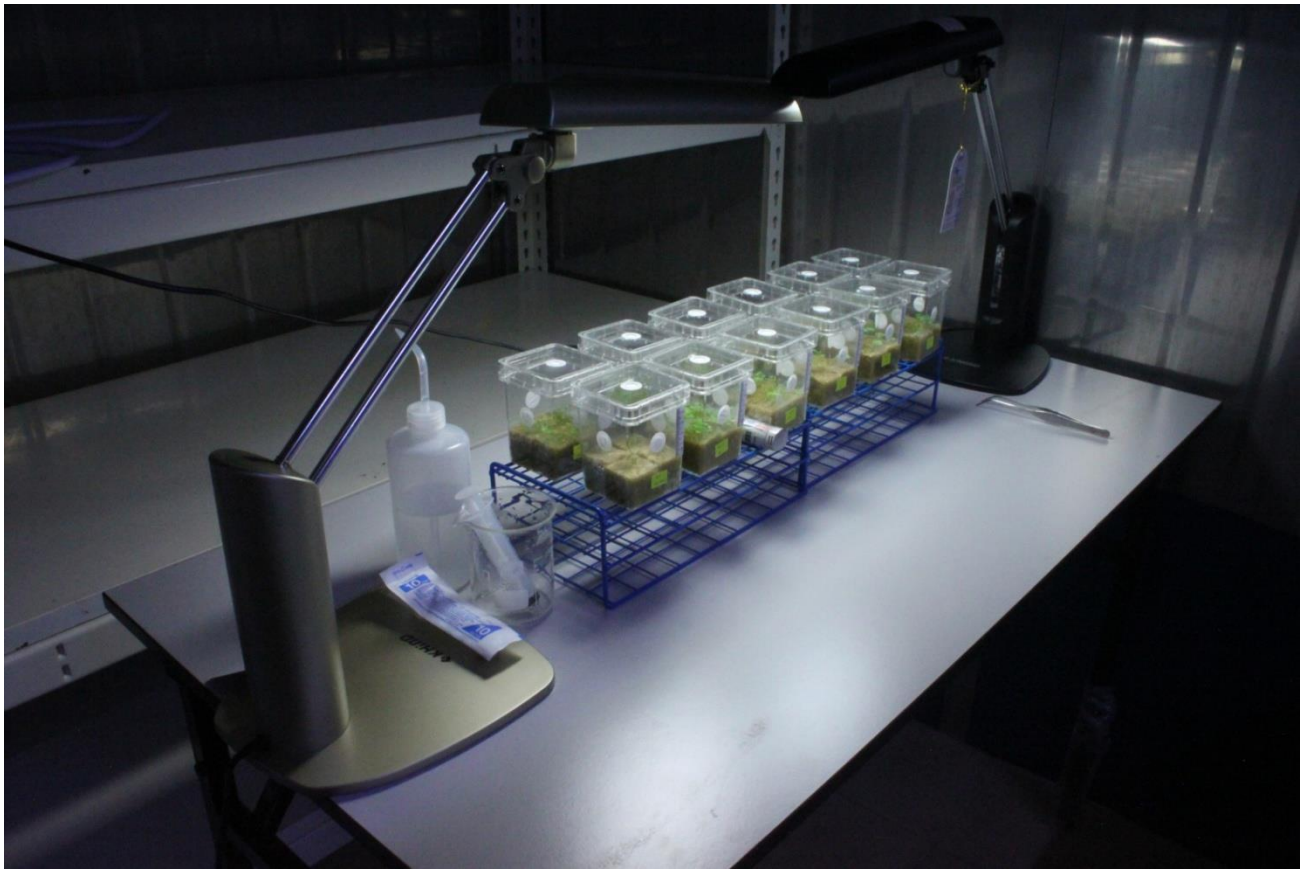
OBJECTIVE

- To study effect on holy basil grow under microgravity environment using gene expression and metabolomic

STATUS ON GROUND EXPERIMENT

- Germination of Holy Basil (*Oscimum Sanctum*) seed
- RNA Extraction
- Prelimenary Microscopic and Macroscopic studies
- Metabolomic Analysis (New)

Germination of Holy Basil (*Oscimum Sanctum*) Seed



Germination room at Gene bank, MARDI

Germination room
Temperature 23-25°C

Light intensity:
40micromol/m^2/sec

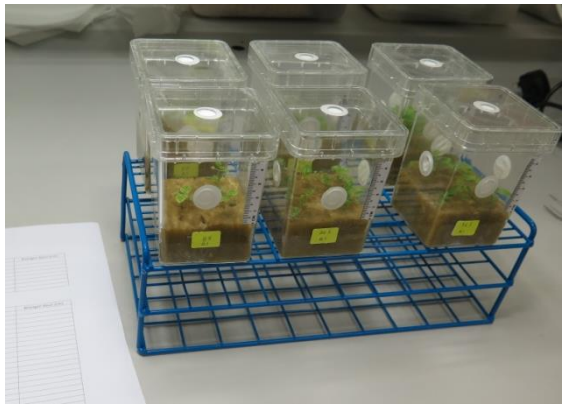
Germination duration:
25 days

Control watering :
100ml -day 0 & 5 and
10 ml for day 10 & 20

Treatment:
- Nutrient (6%N -10%P-5%K)
- 100ml -day 0 & 5 and 10 ml for day 10 & 20

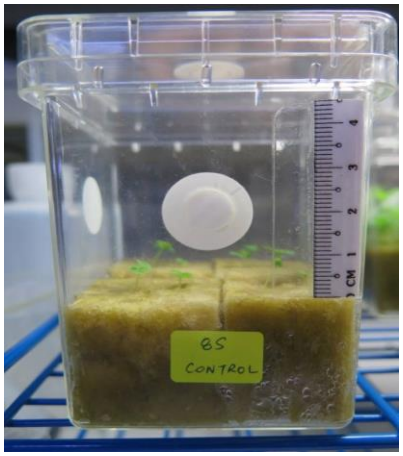
DATA COLLECTION – DAY 25

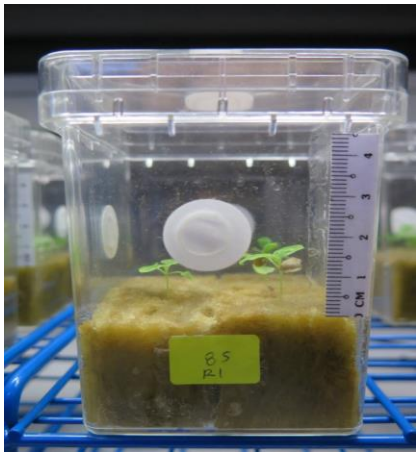
- Seed germination (8, 20,36,64 and 100 seeds)
- Growth data
  a)Seedling height
  b)Leaf size
  c)Leaf number
  d)Seedling fresh weight

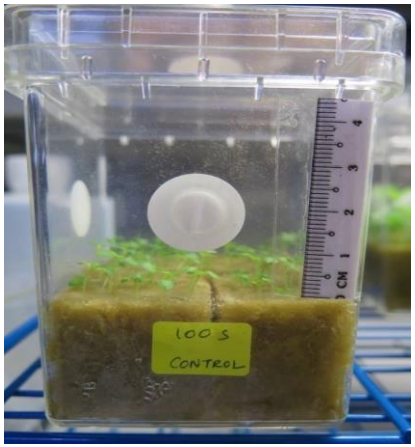


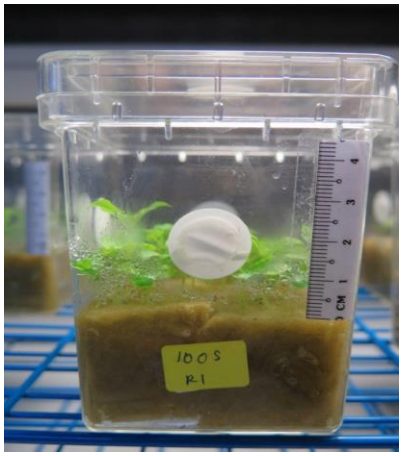
Vigorous growth of Holy basil in nutrient

8 SEEDS		100 SEEDS	
$+H_20$	+ NUTRIENT	$+H_20$	+ NUTRIENT





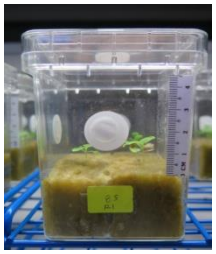




DAY 25

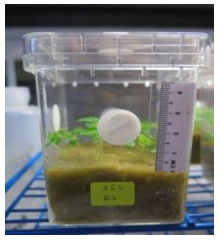
High number of seed produce higher fresh weight

T1 – 8 seeds
Fresh weight:0.105g

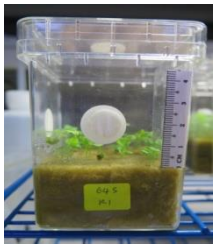


T2–20 seeds
Fresh weight:0.175g

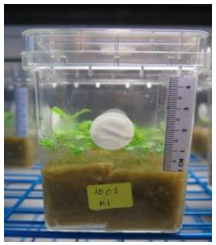
T3 – 36 seeds
Fresh weight:0.325g

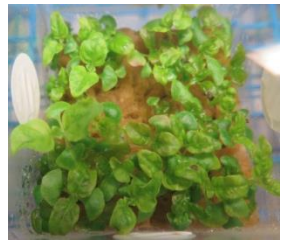


T4– 64 seeds
Fresh weight:0.245g

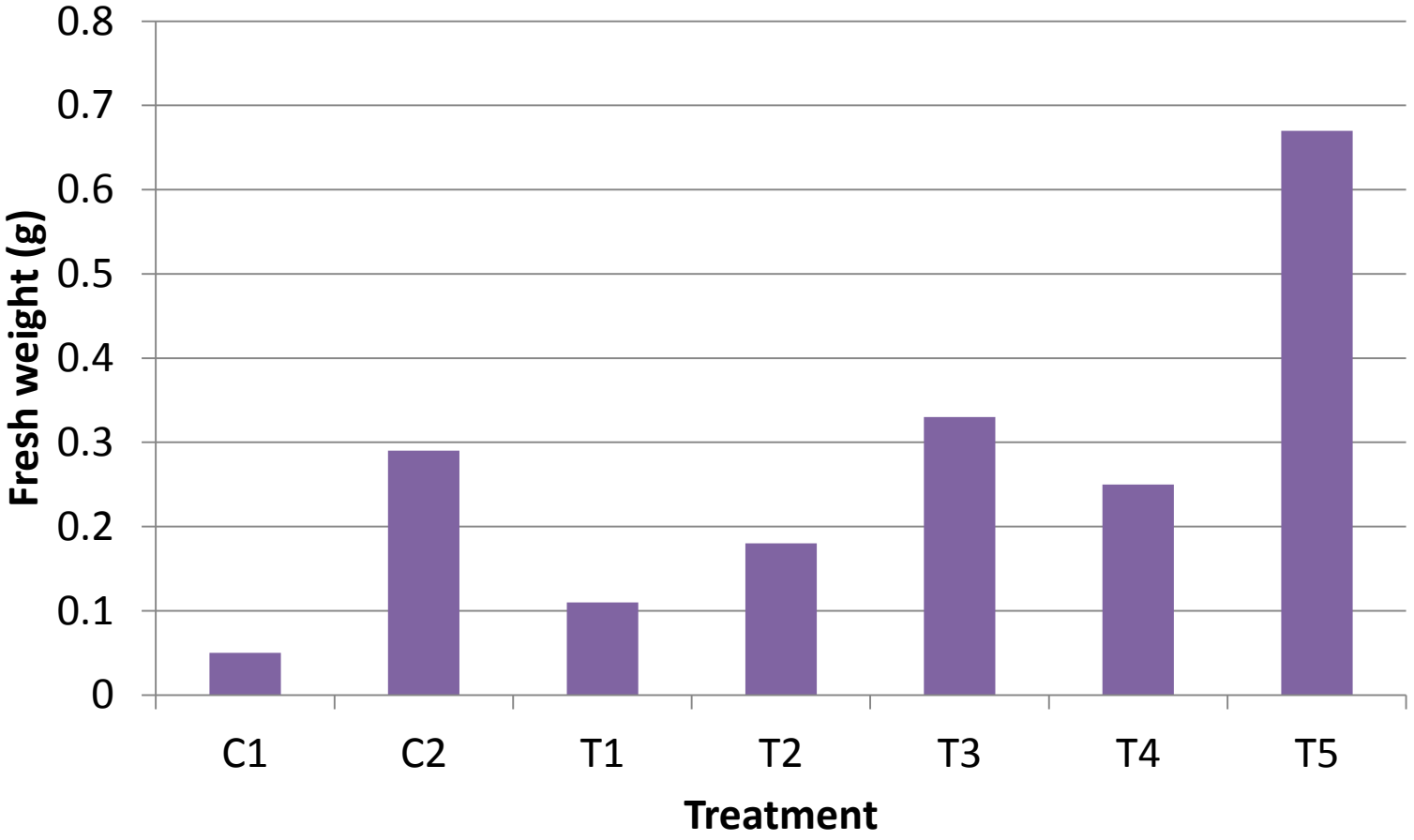


T5 –100 seeds
Fresh weight:
0.67g

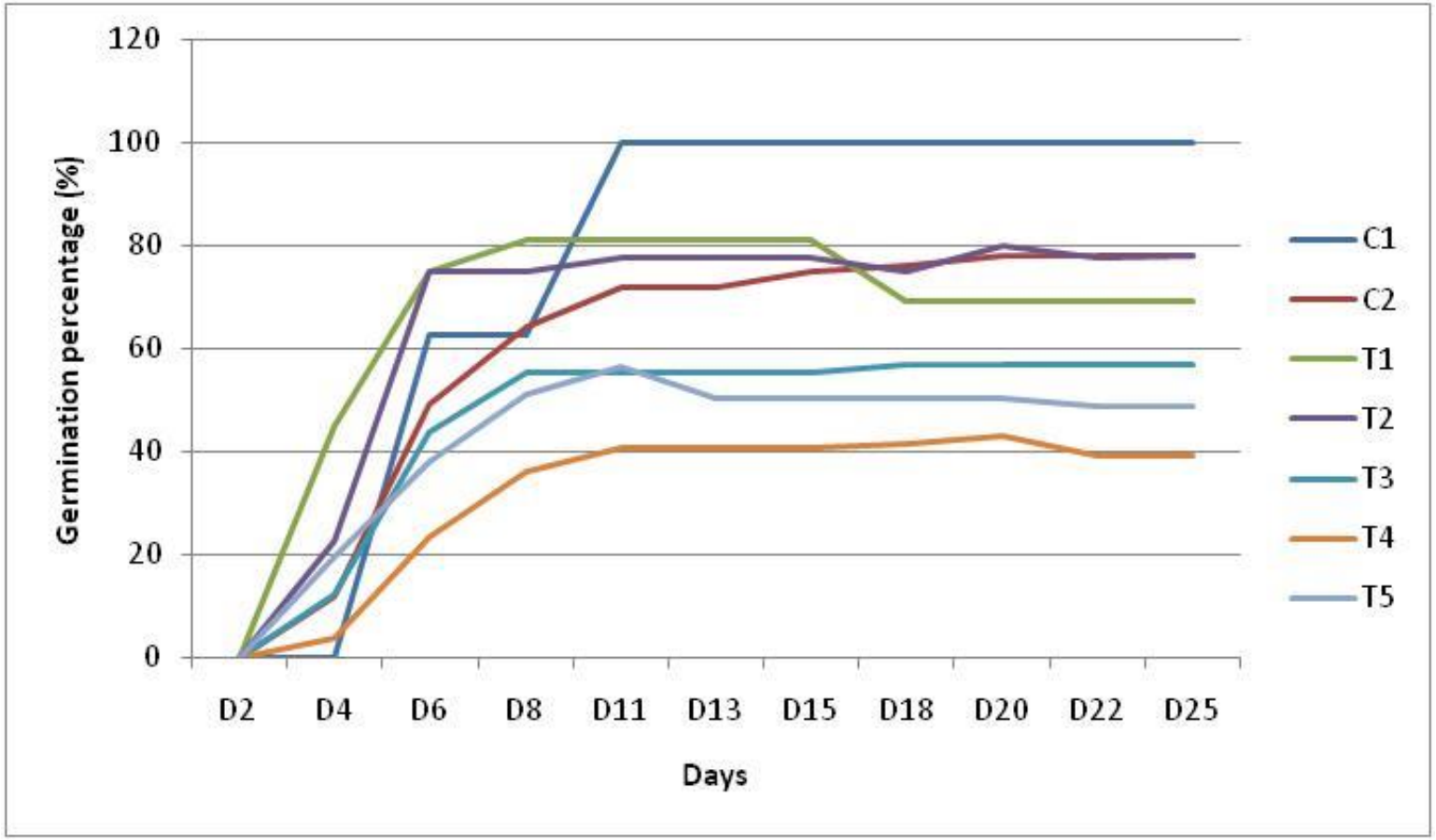




FRESH WEIGHT

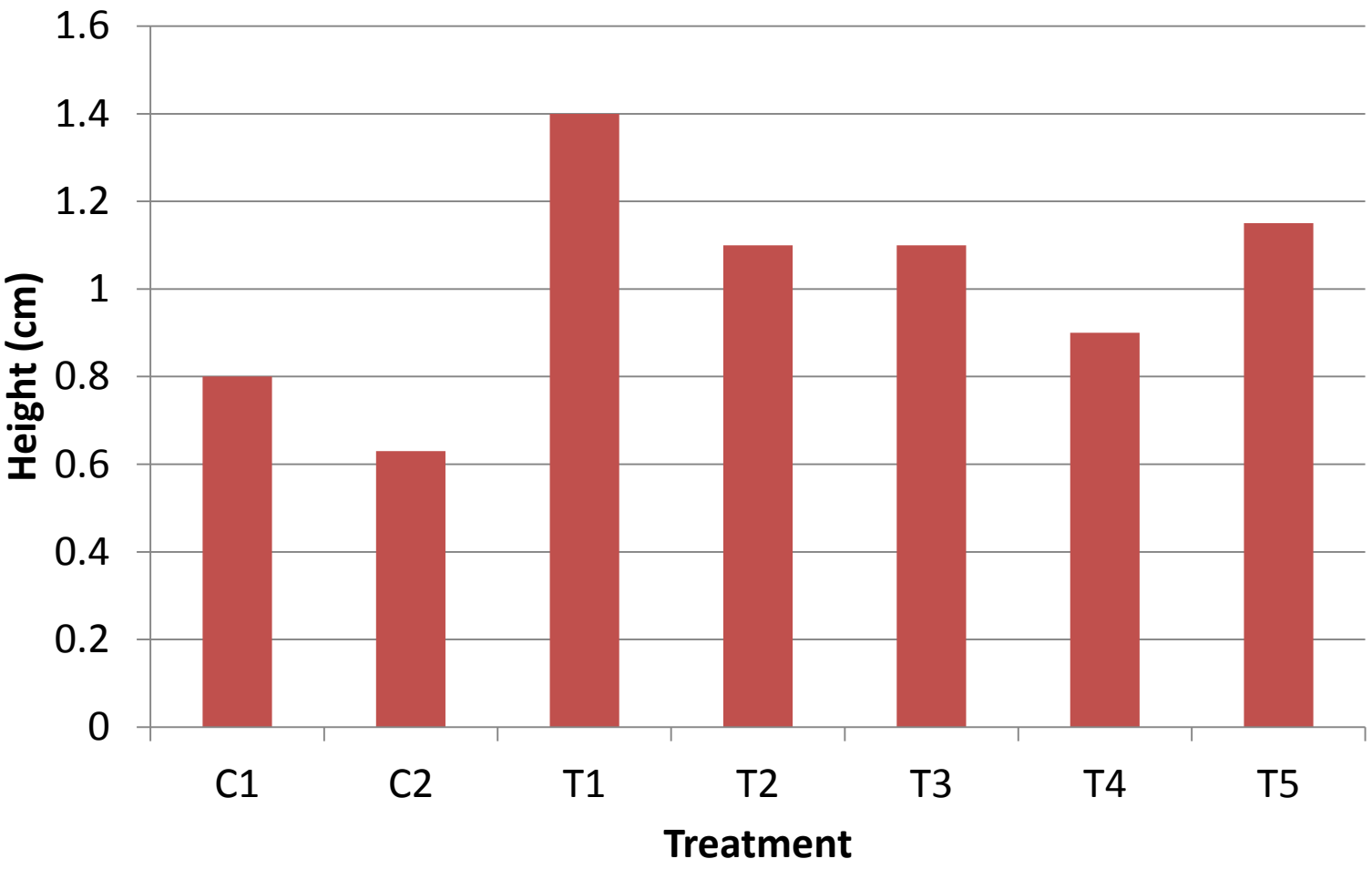


Germination % over 25 days

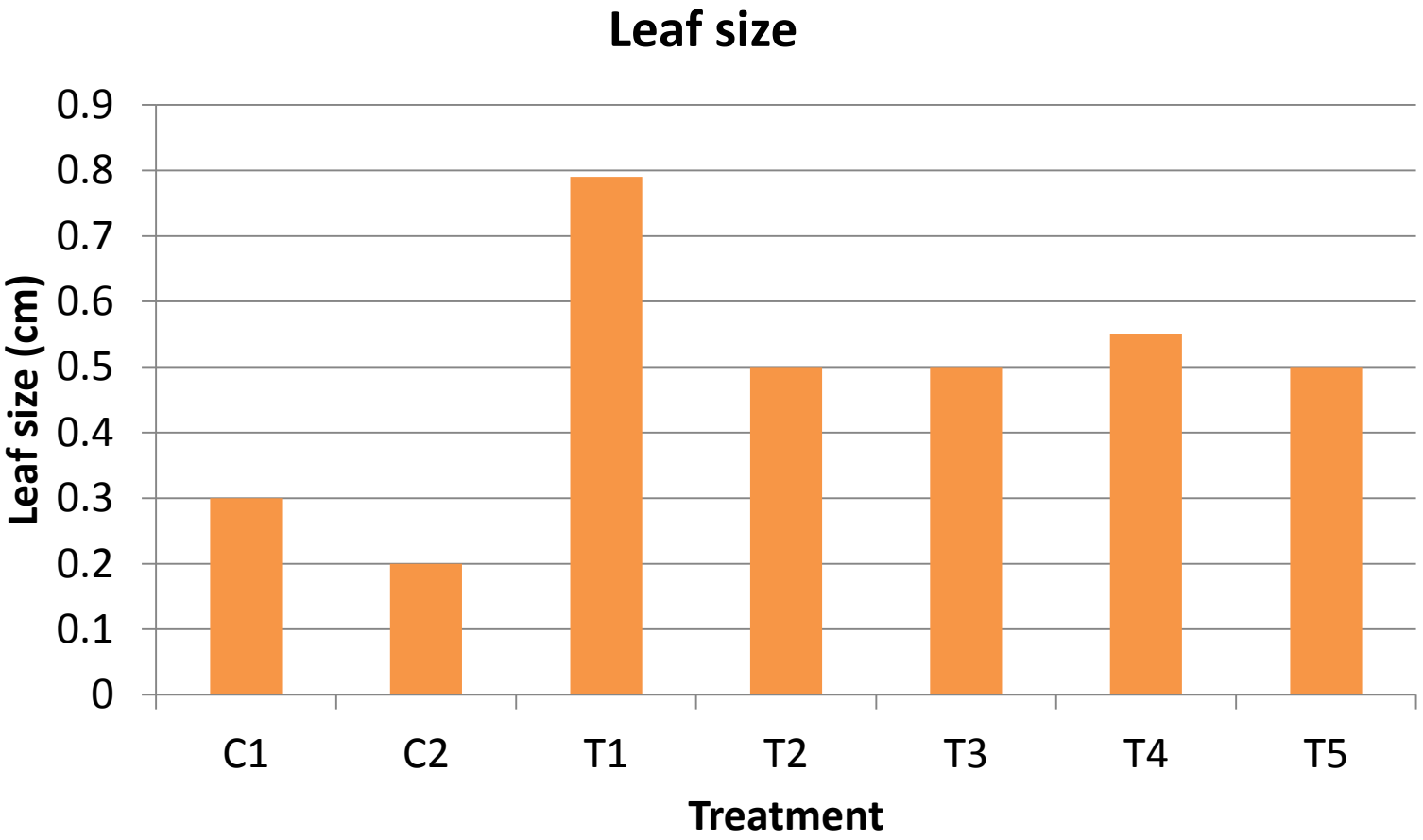


C-Control
T-Treatment with nutrient

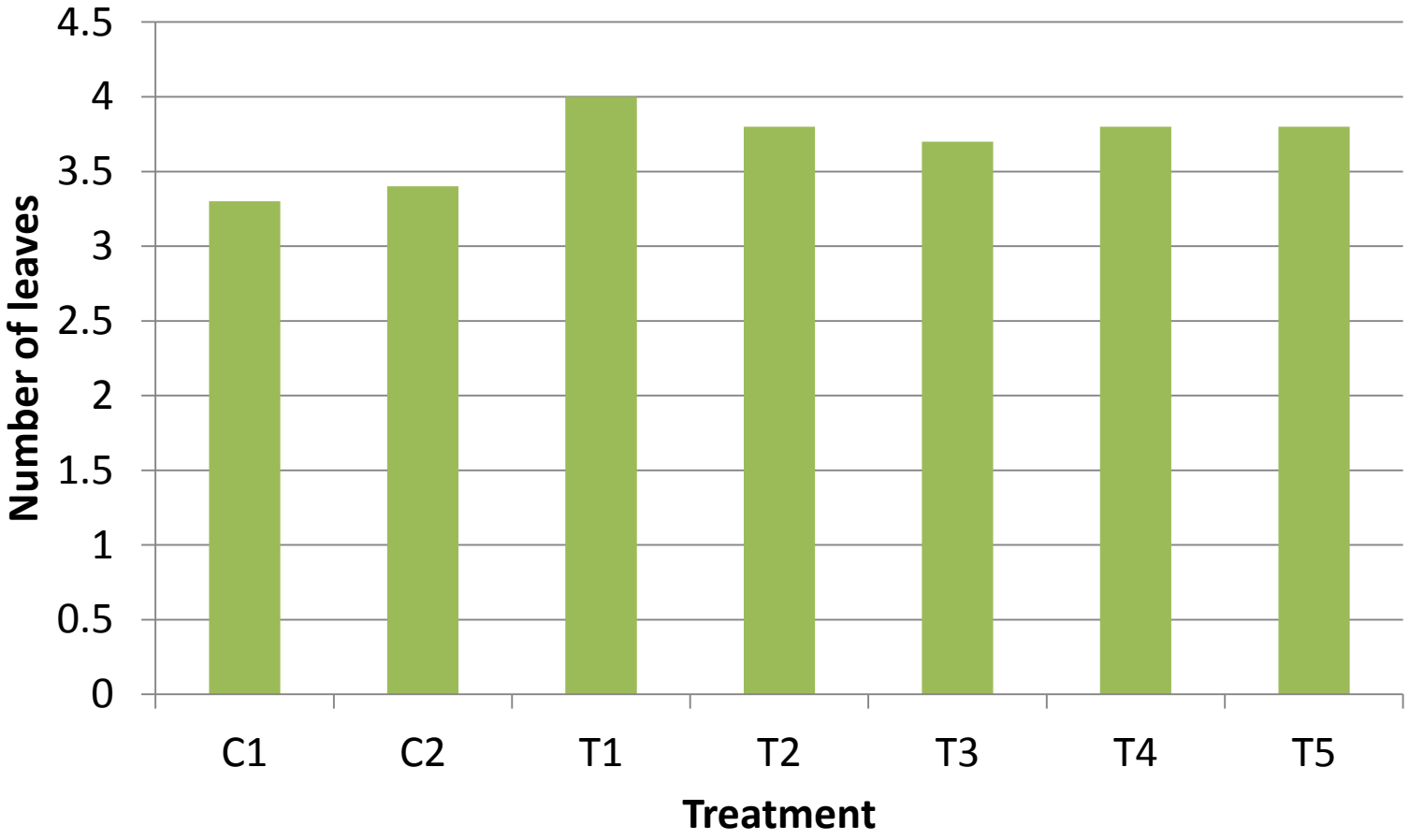
SEEDLING HEIGHT



LEAF SIZE


Leaf size
Leaf size (cm)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
C1
C2
T1
T2
T3
T4
T5
Treatment

LEAF NUMBER


4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Number of leaves
C1
C2
T1
T2
T3
T4
T5
Treatment

STATUS ON GROUND EXPERIMENT

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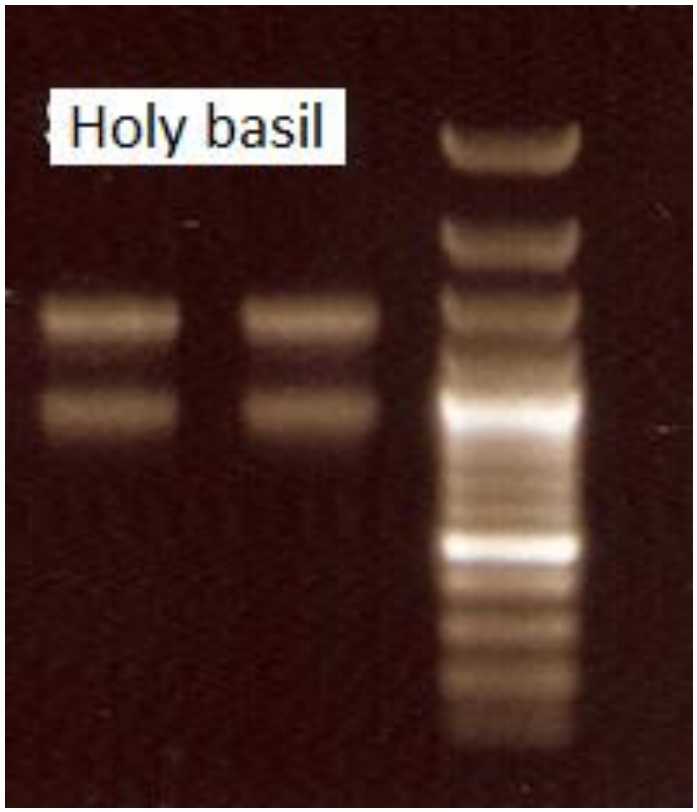
- Metabolomic Analysis (New)

RNA extraction

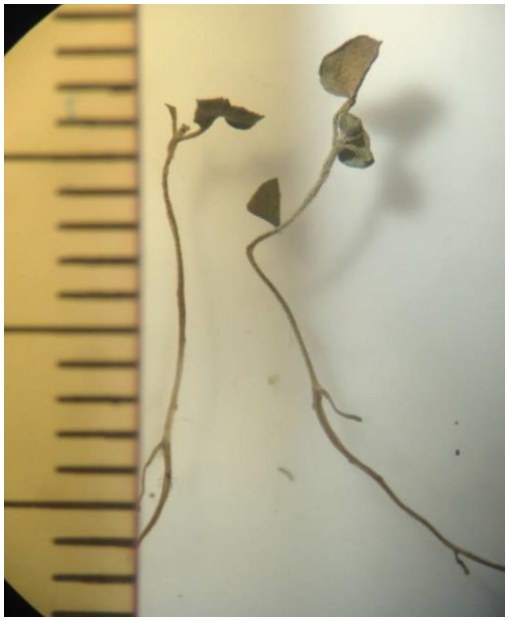


NTES method

Concentration: 154 ng/uL
Total RNA =23 ug (150 uL DEPC water)

Min RNA requirement for gene expression = 10 ug

Prelimenary Macroscopic study

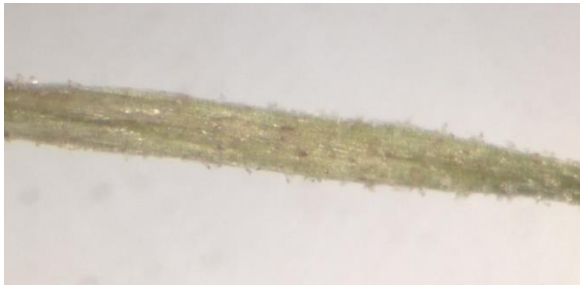


Length

Leaf

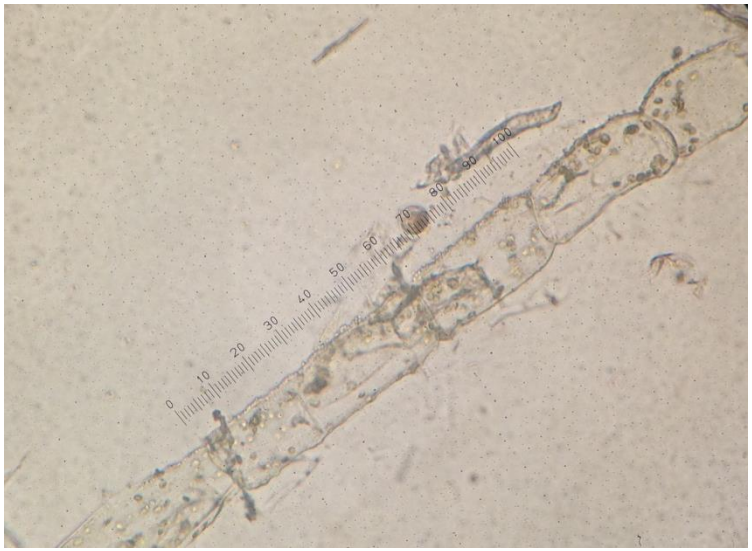
Stem

Macroscopic studies of leaves, stem and root of Holy basil

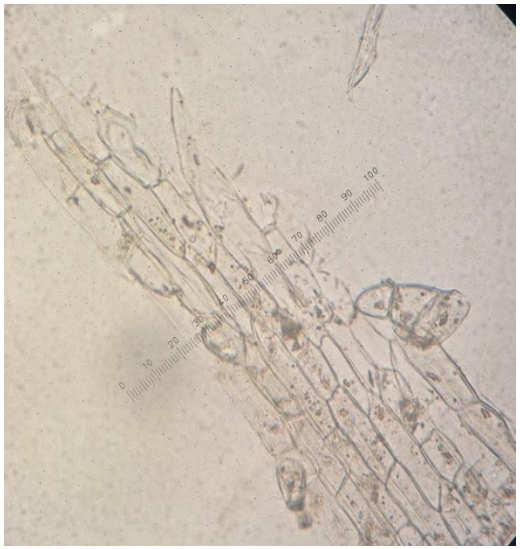


Root

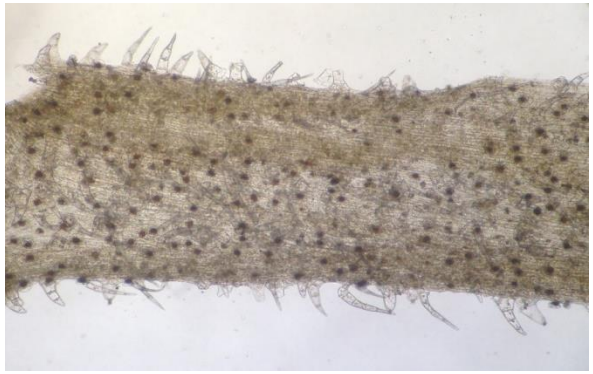
Prelimenary Microscopic study



Stem Cell



Stem Cell



Hairy root

STATUS ON GROUND EXPERIMENT

Germination of Holy Basil (*Oscimum Sanctum*) Seed

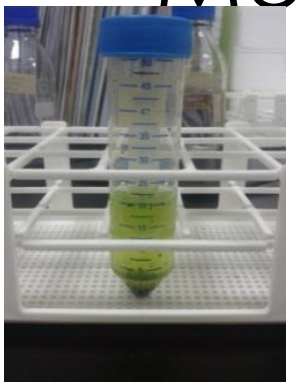
- RNA Extraction

- Metabolomic Analysis

Major Phytochemicals Constituents in Holy basil : Reported Studies

Reported by	Phytochemicals identified	Phytochemicals clusters	Tools used
Shanmuga et al. 2011	Rosmarinic acid, Ursolic acid	Phenylpropanoid and Triterpenoids	HPLC and LCMS
Shanmuga et al. 2013	RA, UA, eugenol and essential oil	Phenylpropanoid, Triterpenoids, essential oils	LCMS
Dutta et al. 2007	Eugenol, luteolin and apigenin	Essential oil, flavanoids	LCMS
Rastogi et al. 2013	Eugenol biosynthesis	Essential oil	Not mentioned
Dev et al. 2010 *other types of basil	Eugenol, isopropyl palmitate etc	Essential oil	GCMS

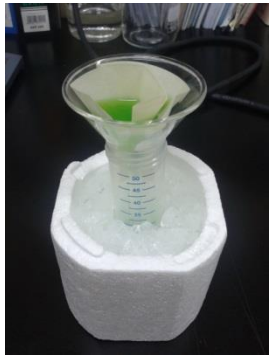
Extraction of Holy basil in Metabolomics Lab, MARDI



Freeze dried Basil (0.1g) + methanol

Vortexed

centrifuged

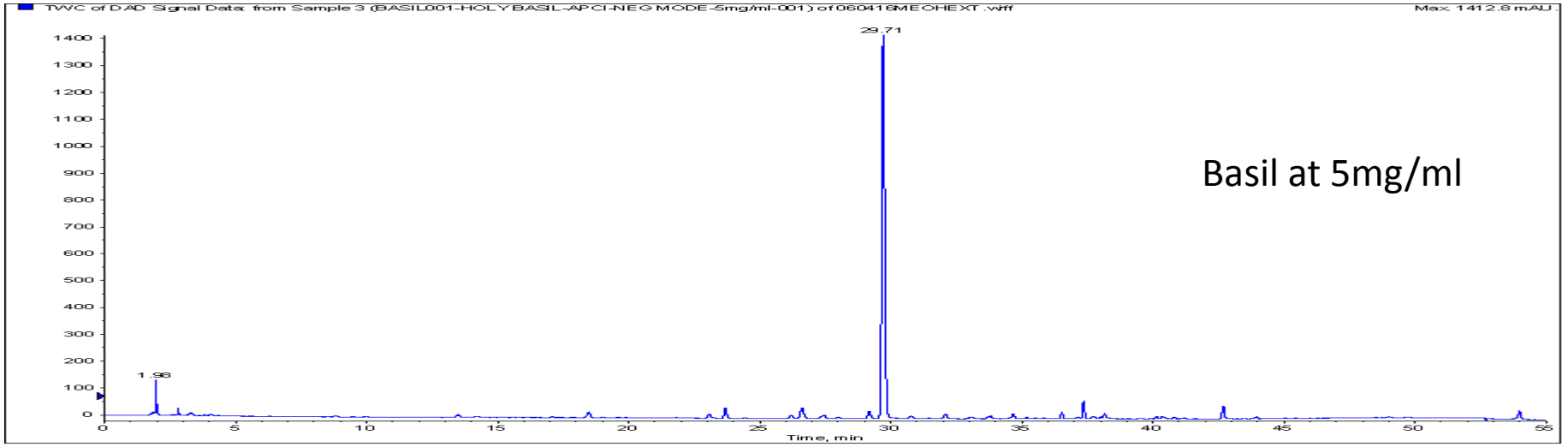


Filtration and collection of supernatant

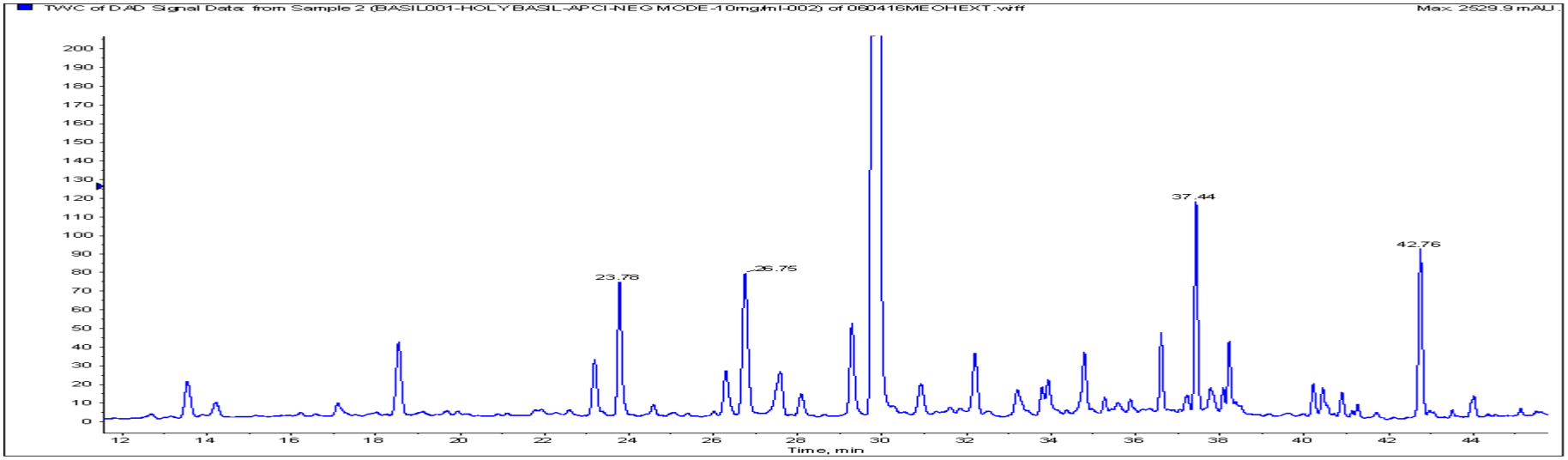
Drying of supernatant

Analysis via LCMS/MS

Chromatogram and Major Phytochemical Constituents in Holy basil



Basil at 5mg/ml



CONCLUSIONS

- Germination of control Holy basil is higher compare to treatment with nutrient
- Germination time of Holy basil treated with nutrient treatments is slower compare to control
- Growth of seedling height, leaf size, leaf no is higher in low seed density due to less competition
- Fresh weight (biomass) is higher in high seed density (T5) due to nutrient efficiency

CONCLUSIONS (cont)

- Necrotic leaves due to nutrient imbalance and deficiency/toxicity
- Established RNA extraction method from Holy basil
- Established extraction method for metabolomic analysis
- Established method for macroscopic and microscopic studies

TEAM MEMBERS

Mohd Helmy Hashim
Mhd Fairos Asillam
Ansor Nadirah Ishak
Zuliakurnia Dewi Nurlisman

Dr. Umarani

Dr.Zulkeflie Zamrood
Mat Noor

Wan Zaki Wan Mamat
Mohd Shaib Jaafar
Dr. Sanimah Simoh
Dr. Farah Farhanah Haron
Nur Asyira Azumi
Mohd Rani Awang
Noor Ismawaty Nordin
Chandradevan a/l Machap
Vigneshwaran a/l poobalasing
Zaitialia Mohlisun
Amyita Witty Ugap
Samsiah Jusoh

THANK YOU