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**MOLECULAR CHARACTERIZATION OF GERMINATION OF DATE
PALM SEEDS MAINTAINED AT MICROGRAVITY IN OUTER PACE
(INTERNATIONAL SPACE STATION)**

Wesam Izzeldeen Khader

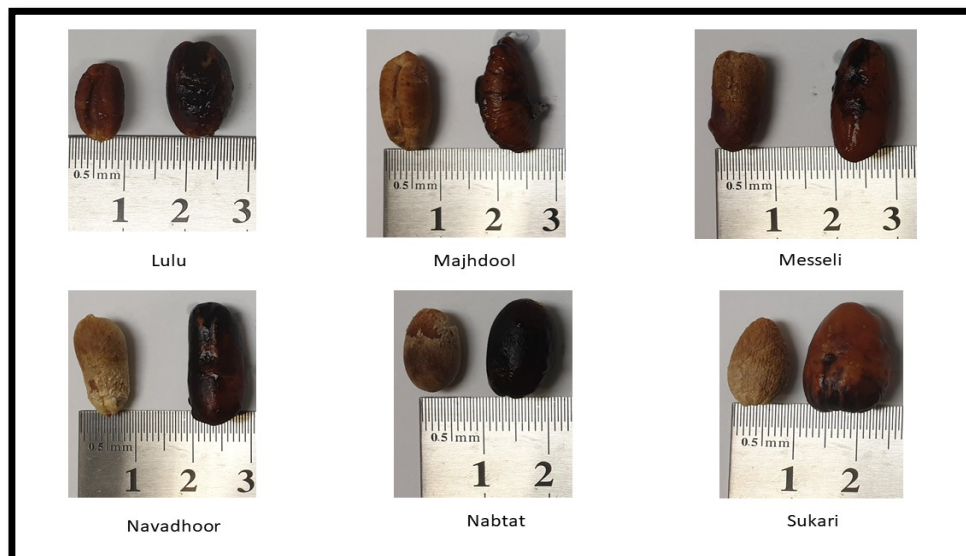
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College of Agriculture and Veterinary Medicine

Department of Integrative Agriculture

**MOLECULAR CHARACTERIZATION OF GERMINATION
OF DATE PALM SEEDS MAINTAINED AT MICRO-
GRAVITY IN OUTER SPACE (INTERNATIONAL SPACE
STATION)***Wesam Izzeldeen Khader**March 2023*

United Arab Emirates University
College of Agriculture and Veterinary Medicine
Department of Integrative Agriculture

MOLECULAR CHARACTERIZATION OF GERMINATION OF
DATE PALM SEEDS MAINTAINED AT MICRO-GRAVITY IN
OUTER SPACE (INTERNATIONAL SPACE STATION)

Wesam Izzeldeen Khader

This thesis is submitted in partial fulfillment of the requirements for the degree of Master
of Science in Horticulture

March 2023

Cover: [Signs of imbibition of water after one month of initiation of the germination experiment in comparison with dry seeds stored at (-) 80°C]

(Photo: By Wesam Izzeldeen Khader & Mr. Shafeeq Rahman)

Declaration of Original Work

I, Wesam Izzeldeen Khader, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled “*Molecular Characterization of Germination of Date Palm Seeds Maintained at Micro-gravity in Outer Space (International Space Station)*”, hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervisor Dr. Shyam Kurup, in the college of Agriculture and Veterinary Medicine at UAEU. This work has not previously formed the basis for the awarded of any academic degree, diploma or a similar title at this or any other university. Any material borrowed from other sources (whether published or unpublished) that is relied upon or included in my thesis has been properly cited and acknowledged in accordance with appropriate academic conventions. I declare that there are no potential conflicts of interest with respect to the research, data collection, authorship, presentation, and/or application of this thesis.

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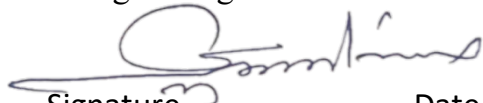


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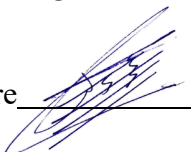
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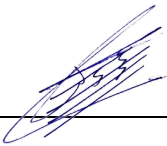

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Abstract

Over the past decade, the UAE has experienced unprecedented innovations in the space sector. Collaborating closely with universities and space agencies around the world, the country's efforts have advanced steadily but quietly under the public radar. The proposed research focuses on basic research to complement the UAE initiative to send date palms to Mars for future space missions. Plant growth analysis of date palm seeds was performed after maintaining at zero gravity in outer space in the International Space Station (ISS) and simultaneously under normal gravity at ground level. In this context, this work was carried out to analyze the molecular changes in the date palm seeds maintained at microgravity in comparison with the gravity seeds. Ninety seeds of different varieties of date palm were sent to space in collaboration with the UAE space agency and the seeds were returned after 6 months. The seeds returned from space did not show signs of germination and, therefore, the molecular mechanism of seed dormancy was investigated with transcriptomics. RNA was extracted from the date palm control and space-maintained seeds and transcriptome analysis was performed. From this analysis, all date palm seeds yielded Q30 values greater than 90%. The GC content of date palm seed samples ranged from 48 to 51%. The higher reads were observed in the Lulu space samples, whereas lower ones were observed in Mesalli seeds. The preprocessed and rRNA-removed reads were used for reference-based pair-wise alignment with the Date Palm NCBI reference genome. We have observed upregulated genes and downregulated genes in all the varieties. The functional profiling of the differentially expressed genes was identified in the Lulu, Majdool and Mesalli date palm seeds. We have found out there are differential gene expressions in all the date palm seeds studied, which might have prevented the seeds from germination. Thus, radiation and vibration damage might have affected both living and non-living components in many ways. We found that these factors affect seeds and their germination process. Also, maintaining seeds in space directly or indirectly affect the overall seed performance by changing the integrity of internal cell organelles.

Keywords: Date Palm, Space missions, Zero gravity, ISS, Transcriptome analysis, Lulu, Majdool, Mesalli.

Title and Abstract (in Arabic)

التوصيف الجزيئي لنمو بذور نخيل التمر المحفوظة في الجاذبية الدقيقة في الفضاء الخارجي (محطة الفضاء الدولية)

الملخص

على مدى العقد الماضي، شهدت الإمارات العربية المتحدة ابتكارات غير مسبوقة في قطاع الفضاء. بالتعاون الوثيق مع الجامعات ووكالات الفضاء في جميع أنحاء العالم، تقدمت جهود البلاد بشكل مطرد ولكن بهدوء تحت الرادار العام. يركز البحث المقترح على البحوث الأساسية لاستكمال مبادرة الإمارات لإرسال نخيل التمر إلى المريخ لبعثات فضائية مستقبلية. تم إجراء تحليل نمو النبات لبذور النخيل بعد الحفاظ على انعدام الجاذبية في الفضاء الخارجي في محطة الفضاء الدولية وفي نفس الوقت تحت الجاذبية الطبيعية على مستوى الأرض. في هذا السياق، تم تنفيذ هذا العمل لتحليل التغيرات الجزيئية في بذور نخيل التمر المحفوظة في الجاذبية الصغرى مقارنة ببذور الجاذبية. تم إرسال 90 بذرة من أصناف مختلفة من بذور نخيل التمر إلى الفضاء بالتعاون مع وكالة الفضاء الإماراتية وأعيدت البذور بعد 6 أشهر. لم تنبت البذور التي تم إرجاعها من الفضاء وللتحقق من المستوى الجزيئي لتحليل ترانسكريبتيوم سبات البذور. تم استخلاص الحمض النووي الريبي من نخيل التمر وأجري تحليل بذور الفراغ والنسخة. من تحليل الترانسكريبتوم، أعطت جميع بذور نخيل التمر قيمًا أكبر من 90%. تراوح محتوى الجوانين-السيتوزين في عينات بذور نخيل التمر من 48 إلى 51%. لوحظت القراءات الأعلى في عينات الفضاء اللولو بينما لوحظ انخفاض في عينات الفضاء ميسالي. تم استخدام قراءات حمض الريبوسوم المزالة والمعالجة مسبقًا من أجل المحاذاة الزوجية المستندة إلى المرجع مع الجينوم المرجعي للمركز الوطني لنخيل التمر لمعلومات التكنولوجيا الحيوية. لقد لاحظنا الجينات المنتظمة والجينات المنتظمة في جميع الأصناف. تم التعرف على التنميط الوظيفي للجينات المعبر عنها تفاضليًا في بذور نخيل اللولو، مجدول ومسالي. لقد اكتشفنا أن هناك تعبيرًا جينيًا مختلفًا في جميع بذور نخيل التمر التي تمت دراستها والتي قد تمنع البذور من الإنبات. وبالتالي، يؤدي الإشعاع والاهتزاز إلى إتلاف كل من المكونات الحية وغير الحية بعدة طرق. وجدنا أن هذه العوامل تؤثر على البذور وإنباتها. أيضًا، يؤثر السفر في الفضاء بشكل مباشر أو غير مباشر على الأداء الكلي للبذور من خلال التأثير على عضيات الخلية الداخلية.

مفاهيم البحث الرئيسية: وكالة الفضاء، بذور النخيل، الجاذبية الصغرى، تحليل التغيرات الجزيئية، تحليل الترانسكريبتوم، لولو، مجدول، ميسالي.

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I would like to express my hearty, deep sense of gratitude and sincere thanks to Dr. Shyam Kurup for his periodical guidance in planning and execution the work. I also would like to thank my committee for their guidance and support throughout the years.

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To my beautiful sisters in law Maryam and Rahaf, your support and prayers are unforgettable. Thank you!

I also express my gratitude to the CAVM for providing the research infrastructure to carry on the work.

Dedication

I would like to dedicate this thesis to my family. Thank you so much for everything! Words can hardly describe my thanks and appreciation to you. You have been my source of inspiration, support, and guidance. You have taught me to be unique, determined, to believe in myself, and to always persevere. I am truly thankful and honored to have you as my family.

Table of Contents

Title.....	i
Declaration of Original Work.....	iii
Approval of the Master Thesis.....	iv
Abstract.....	vi
Title and Abstract in Arabic.....	vii
Acknowledgments	viii
Dedication.....	ix
Table of Contents.....	x
List of Figures.....	xii
List of Tables	xiii
List of Abbreviations	xiv
Chapter 1:Introduction.....	1
Chapter 2: Literature Review.....	5
2.1 Harsh Environment and Stress Tolerance	6
2.2 Space Farming.....	7
2.2.1 How Plants Grow in Space	7
2.3 Gravitropism and Phototropism	9
2.3.1 Other Tropisms that may be Implicated in Response to Altered Gravity Levels	10
2.4 Effects of Microgravity on Plants.....	10
2.4.1 Disrupted Meristematic Competence.....	10
2.4.2 Cell Cycle.....	11
2.4.3 Transcriptomic Changes Under Microgravity and Spaceflight	11
2.5 Constraints of Space Research	12
2.5.1 Simulated Microgravity	13
2.6 RNA Role in Seed Germination.....	13
2.7 Phytochromes Inhibit Hypocotyl Negative Gravitropism.....	15
2.8 Singlet Oxygen-mediated Programmed Cell Death	16
2.9 Anaerobic Respiration: The Key to Germinate Under Oxygen Deficiency.....	17
2.10 Role of Gibberellic Acid and Other Hormones in Seed Germination.....	17

2.10.1 Seed Germination.....	19
2.10.2 Seed Dormancy	21
2.10.3 ABA	22
2.10.4 Ethylene	23
2.10.5 Cytokinins	23
2.11 Effect/Problems of Microgravity on Plants.....	24
2.12 Effect of Radiation on Plants.....	26
Chapter 3: Materials and Methods.....	29
3.1 Seed Material.....	29
3.2 Seed Germination	30
3.3 Germination of Space-maintained Seeds in Comparison with Gravity Seeds Using GA and Cytokinin	30
3.4 Transcriptome Studies	30
3.4.1 RNA Extraction.....	31
3.5 Bioinformatics Analysis Pipeline.....	31
3.5.1 Read Quality Check	31
3.5.2 Preprocessing and rRNA Removal	32
3.5.3 Read Alignment	32
3.5.4 Differential Expression Analysis	32
Chapter 4: Results.....	33
4.1 Germination Experiment on Microgravity Seeds and Gravity Seeds	33
4.2 RNA Extraction	34
4.3 Data Summary	35
4.4 Alignment with Date Palm Genome	35
4.5 Differential Gene Expression Analysis	36
4.6 Differential Expression FPKM Plot	37
4.7 Functional Profiling of Differentially Expressed Genes	40
Chapter 5: Discussion	43
Chapter 6: Conclusion	48
References.....	49
Appendix.....	62

List of Figures

Figure 1: Bioinformatics Analysis Pipeline.....	31
Figure 2: Signs of imbibition of water after one month of initiation of the germination experiment in comparison with dry seeds stored at (-) 80°C.....	33
Figure 3: QC analysis of Mesalli sample using the CTAB trizol method. 18s and 23s subunits of RNA were clearly visible in this method with good RNA concentration.....	34
Figure 4: FPKM plot and volcano plot of the Lulu control vs. Lulu space differential expression.....	37
Figure 5: FPKM plot and volcano plot of Majdool control C vs. Majdool space differential expression.....	38
Figure 6: FPKM plot and volcano plot of Mesalli Control vs Mesalli Space differential expression.....	39
Figure 7: Summary of the results obtained with a p-value threshold of 0.05 between the G profiler for the Lulu control and lulu space.....	40
Figure 8: Summary of the results obtained with a p-value threshold of 0.05 between the G profiler for Majdool control and Majdool space. The intracellular anatomical structure (GO:0005622) was among the top log values.....	41
Figure 9: Summary of the results obtained with a p-value threshold of 0.05 between the G profiler for Mesalli control vs. Majdool space. Cytosolic ribosomes (GO:0022626) were among the most significant results.....	42

List of Tables

Table 1: Raw FASTQ summary of date palm seeds after sequencing	35
Table 2: Read alignment summary of date palm seeds after RNA seq.....	36
Table 3: Differentially expressed genes (p-value \leq 0.01).....	36
Table 4: Differentially expressed genes (p-value \leq 0.05)	37

List of Abbreviations

ABA	Abscisic Acid
APH	Advanced Plant Habit
GA	Gibberellic Acid
G1	Growth Phase 1
G2	Growth Phase 2
HSP	Heat Shock Proteins
LED	Light-Emitting Diode
PCD	Programmed Cell Death
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
UV	Ultra-Violet

Chapter 1: Introduction

The date palm tree is regarded as one of the oldest and most ancient crops in North Africa and Southwest Asia. It can also be grown in various countries such as Australia, Mexico, South America, and the United States (Chao & Krueger, 2007). The date palm tree belongs to the *Arecaceae* family, which includes over 2,500 species and approximately 200 genera. One of the genera that is commonly found in these regions is the *Phoenix* (Yahia et al., 2013). This plant is native to either subtropical or tropical regions in Africa or Asia. *Dactylifera* is a group of date palm tree species that includes the Greek word *dactylus*, meaning finger, and the Latin word *ferous*, meaning bearing (Ashraf & Hamidi-Esfahani, 2011). The flowers of the date palm tree are yellow and small, and they are attached to spikelets that develop into fruits called date fruits (El Modafar & El Boustani, 2001). Due to the fast-growing demand for this product, the production of dates has increased significantly over the years. In 1990, the world's 20 top date-producing countries had a combined production of around 3.5 million metric tons. Ten years later, the production of these countries had increased by almost double. The whole genome of this plant was recently re-sequenced to gain insights into the diversification of its fruit tree crop.

The seeds of date palm fruits are enclosed by a fibrous material known as the endocarp, fleshy mesocarp, and fruit skin. Different regions that produce dates have varying shapes and sizes. In addition, their organoleptic, physical, and chemical characteristics vary. Although they are usually oblong in shape, certain varieties of fruit can also reach a spherical shape. The date palm bears fruits at around five to six years old. It can produce up to 600 kilograms per year for up to 60 years. Some of the countries that are the top ten date-producing nations include Iran, Iraq, Pakistan, Egypt, Saudi Arabia, and UAE. Around 100 million date palm trees are scattered across 1.3 million hectares globally. The biggest contributor to the production of dates is the Asian continent, with over 900,000 hectares, followed by Africa, with over 400,000 hectares (Al-shahib & Marshall, 2003).

Considering the importance of the date palm across the world, this study assumes great significance in space farming and future Mars programs. Plant science research in

space has shown innovative mechanisms and the potential of dry seeds, growing seedlings and flowering plants to respond to environmental change and harsh conditions, including on orbiting space platforms such as the International Space Station outside their evolutionary history. These discoveries have implications for sustainable agriculture, climate change, food security, and seed storage on the Earth's surface, as well as for growing fresh food during long-distance space flights and agriculture on Mars and other worlds. Laboratories aboard spacecraft in low Earth orbit have allowed researchers to investigate the impacts of long-term microgravity. For example, uncovering mechanisms behind plant tropism that would otherwise be obscured by gravitropism effects. Epigenetics, transcriptomics and proteomics (Link et al., 2014) investigation of growing seedlings and mature plants in microgravity discovered known and novel genes specific for the response to the space environment. This discovery could be used to cultivate fresh food derived from plants in space for human nutrition and survival during long-distance space travel. These studies showed that successful 'seed-to-seed' plant culture and multiplication is achievable but that the reserve content and qualities of space-produced seeds can change. Long-term microgravity exposure inside spaceships has led to the crucial revelation that it is linked to accelerated aging in people and plants.

Plants stand all around us, but in what way do they grow and what makes them grow? Plants want various things to grow, such as water, air, nutrients, light, space temperature, and time. Like humans and other living things, plants need water and nutrients to grow. Most plants require to transport water and nutrients between roots and leaves. As a food supplement, water is usually absorbed from the soil by the roots. Therefore, it is significant to water plants once the soil dries out. Fertilizers are also a supplement for plants and are often administered when watering plants. The important supplements for plant growth are nitrogen, potassium and phosphorus. Nitrogen is important for the formation of green leaves, phosphorus is necessary for the creation of large flowers and strong roots, whereas potassium supports plants to avoid infections. Too tiny or too much water or food supplements can also be detrimental (Bouguedoura, 2015).

Apart from water and supplements, fresh and clean air and healthy soil are vital for growing plants. Polluted air, caused by smog, exhaust fumes, and various toxins, is dangerous for plants and binds to carbon dioxide in the air for food production (photosynthesis). Although basic additions of natural materials and microorganisms originate in the soil, they offer an anchor for rooting and support for plants. Plants need daylight to grow, which works as an energy source to produce food, a process known as photosynthesis. Also, their flowers and natural products will be reduced. Most plants tend to have a lower temperature at night and a higher temperature during the day. If it is too hot, they will tire; if it is too cold, they will freeze (Tripler, 2011) .

Reduced gravity alters the plant's physical environment, which in turn affects physiological water and solute transport, as well as gas exchange between the plant and its surroundings (Porterfield, 2002). Cosmic radiation, on the other hand, alters gene expression levels and has an impact on the genome through DNA damage and chromosome alterations (Baumstark-Khan et al., 1999). However, the effects do not appear to be deleterious to plant growth and survival at this time (Karoliussen et al., 2013). Although plants have been grown in low Earth orbit for numerous generations, it is still unknown whether the plant genome will remain stable in space. Long-term low-dose of chronic radiation is considered to be more important than high-dose acute radiation. Furthermore, persistent low-dose ionizing radiation exposure has been demonstrated to have a larger effect on plant genetics than an acute dose (O. Kovalchuk, 2000). Chronic radiation exposures have been the subject of very few research (Real et al., 2004). Chronic ionizing radiation appears to have a long-term effect on the population genetic structure, and a reduction in genetic variability may be an adaptive response to chronic stress (Esnault et al., 2010). The reaction to chronic or acute radiation exposure is mediated by many pathways (I. Kovalchuk et al., 2007). While oxidative stress-related genes are the well-represented group of genes affected by acute radiation exposure, chronic stress causes a completely distinct response that is reflected in adaptive responses by regulating genes associated with general stress and nucleic acid metabolism. Several genes involved in photosynthesis and carbohydrate metabolism are also induced by chronic stress (I. Kovalchuk et al., 2007). A magnetic field on top of a geomagnetic field has been used in several studies to show that it influences plant

development and photosynthesis (Ahmad et al., 2007). According to both types of studies, changes in magnetic fields may affect plant growth and development.

Therefore, the present study is carried out to understand the molecular mechanisms associated with seed germination and the impact of microgravity on seed germination in monocot trees like the date palm.

Chapter 2: Literature Review

Humans are constantly weighed down by gravity. The goal of weightlessness is to achieve a functional status that is referred to as simulated microgravity. Various methods have been used to achieve this, such as using a clinostat, magnets, or random positioning machines (Herranz et al., 2013). The goal of these devices is to allow biological systems to adjust gradually to the effects of gravity by exposing them to their influence for a certain amount of time. If the effects of the gravity vector change constantly, the object loses its sense of direction. This behavior mimics the one observed under real microgravity conditions.

Various types of spacecraft, such as satellites, rockets, and drop towers, can be used to achieve real microgravity. However, due to the limited amount of time that these devices can be used, it is not yet clear how they can affect the system's response to the environment. One of the possible effects of microgravity on the system response is the elevation of calcium levels. This is a controversial issue because it affects plant reorientation. The effects of microgravity on the response of the system include a reduction in the mitotic index and proliferation rate of meristematic root cells (Herranz et al., 2010) . Another target of the effects is the cell wall. In rice, the cell wall was shown to be reduced in thickness and had increased elasticity (Hoson et al., 2003). Some plant species, such as mung beans, have shown changes in their lignin levels because of gravity. However, these changes were not observed in other species. Despite the presence of various changes in photosynthetic activity, such as a reduction in the light-harvesting apparatus, direct measurements of these activities did not reveal any changes. One of the possible reasons why the results of the studies were not able to provide conclusive answers is the presence of photosynthesis. In microgravity, the lack of airflow can lead to the accumulation of volatiles and the alteration of gas exchange.

Scientists have used a variety of tools known as clinostats to simulate the effects of microgravity on plant development. One of these is a rotating device known as a clinostat, which constantly changes the direction of the plants to prevent the growth hormone, auxin. If the device is used properly, it can cause roots and stems to grow toward one another, rather than upwards. Since 1879, the use of a clinostat has been used

to simulate the effects of gravity and light on the development of plants (Vogt et al., 2012). A clinostat prevents plant growth owing to gravity. A low rotating speed minimizes the gating effects on the subjects. Some models are inclined, while others can shift their three axes to create random effects. It can also imitate low gravity. By altering the rotation, speed and inclination, plants can be grown in optimum conditions. The effects of a clinostat on plant growth can be mimicked in space. Cell components travel randomly in microgravity, mixing in the cytoplasm. This research reveals how plants adapt to varied gravitational circumstances.

The date palm is considered the most widespread natural product of the developed trees. It is a well-known tree found in desert oases. The date palm is considered a symbol of desert life because it is more resistant to heat, drought, and salt than many other plants. *Phoenix dactylifera* is believed to have originated in the countries surrounding the Persian Gulf in the Middle East, where it developed at least 6,000 years ago. Date palm is found in more than 40 countries. The annual global production of dates is approximately 7.52 million tons in an area of 1.15 million hectares. Middle Eastern countries produce 5.4 million tons per year, accounting for 70% of the world's date palm production (Alawar et al., 2009).

Some special organic characteristics make date palm an interesting natural product tree. The Phoenix can reach more than 20 meters in height and is more than 100 years old (El Rabey et al., 2016). The leaves (palm fronds) of the date palm are in one piece and can move like a heat sheath; therefore, the old leaves must be physically removed during development. Date palms also thrive in harsh conditions, such as long dry spells, high salinity, and high temperatures.

2.1 Harsh Environment and Stress Tolerance

Plant species vary in their resistance to abiotic stress. Date palm varieties can resist salt and drought stress in different ways. The study about how plants vary in their resistance to abiotic stress was conducted to investigate the retort of date palms to high salt and drought concentrations in terms of leaf proteome. 18-month-old date palm plants were exposed to extreme salt and drought conditions for one month. The salt and drought

stress qualities of the harvested protein patches were over-expressed or reported as high or low protein excesses, respectively (Yaish & Kumar, 2015).

2.2 Space Farming

Space agriculture is the cultivation of food and other materials in space or on celestial bodies beyond Earth, much like gardening on the moon. The potential applications of this technology are numerous, ranging from the formation of independent teams for largely supervised trips to Mars to the establishment of a nursery on the International Space Station to the growth of food in space to reduce the harmful environmental effects of agriculture on Earth on nearby biological systems (Ray et al., 2019).

In addition, innovations from space agribusiness developments could be used to improve horticulture on Earth. For example, space travelers should use human excrement to process crops, such as lettuce (Monje et al., 2020). Innovations and facilities made sterile could be transferred to farms in large cities. Closing the energy cycle in this manner and reducing the dependence on chemical compounds would benefit local farms.

2.2.1 How Plants Grow in Space

In planning long-term missions to the Moon and Mars, NASA had to find a way to maintain equipment in space for weeks, months, or even very long periods. NASA wants to develop new plants that are not difficult to produce and do not require additional equipment or expensive electricity. Here are some components of plant development that we have observed on the space station. The first one is choosing the right plant; what grows well on Earth can also thrive in space. Before sending crops into space, researchers must decide which plants to be tested at the space station (Heiney, 2019). To study this cycle, NASA launched a collaboration with Miami's Fairchild Botanical Garden in 2015 called Exploitation beyond Earth. As part of the program, more than 230 high school science classes from across the United States were asked to use extraordinary equipment to develop different seeds. Secondly, discovering how plants grow in Space, plants need a place to grow and NASA has tried to create a microgravity garden at several institutions. One way to try something different is with

the 'Veggie' plant production system, a simple, low-powered growth chamber that can hold up to six productive plants. Teams tend to water the plants by hand as if they were a herb garden on the ground.

The third important aspect is the correct light; the combined effects of light on plants can influence their size, nutrient content, microbial augmentation, and taste. Plants depend on red and blue light for their development. Experts have conducted tests on the space station to observe how diverse ratios of red and blue light affect the expansion of plants in space. The tests demonstrated that plants perform well in space under light conditions like those on Earth. The fourth aspect is the effect of gravity, and accompanied changes in gravity that can influence the development of plants and the amount of fruit they produce. Plants can detect gravity with an instrument that senses changes in the calcium in their cells. Clarified that space scientists aboard space stations had conducted experiments to quantify the importance of microgravity on calcium levels, which could provide a signal for planning better ways to grow food crops in space.

The fifth factor was water transport. A key test for growing plant life in microgravity is to offer a substrate with enough water to remain strong without smothering it with too much water. To this end, several techniques have been tested in various tests, including the "Exploring Water Management in Plants" test. The sixth factor is human impact. Gardens require natural care, such as astronauts or robots, to care for the plants growing in space. NASA has studied the growth in space that can contribute to the activities and health of astronauts. One problem is weightlessness. Although plants can be prevented from floating upward, water and air generally circulate evenly around Earth, making it difficult for plants to access. On the ground, weak air currents can suffocate plants. Organisms are also essential for secreting biomass and providing supplements and CO₂ (Heiney, 2019).

Crop development under false lights is an advanced practice in logical husbandry. Initially, bright lights were used in vertical barns to encourage crop development. However, with the advancement of LED light innovations, bright lights are gradually being replaced by new energy-saving lights. It is preferable to use pink lights, such as a

mixture of red and blue LED lights. Researchers claim that the mixture of red and blue is the only thing that can develop crops with similar flavors.

Seed germination is a vital cycle that affects crop yield and quality. Therefore, understanding the atomic composition of seeds during germination and desiccation is important for improving crop yield and quality (Souza et al., 2017).

2.3 Gravitropism and Phototropism

Plants are known to show a reaction when they respond to external stimuli. This is called tropism; it could be positive tropism, which means the growth is going toward a stimulus, or negative tropism, if it is not growing toward the stimulus. Tropism allows plants to grow toward light or roots toward the water found in the soil (Muthert et al., 2020). Gravity is a unique and very important factor that instructs plant roots to grow toward the water; this is called positive gravitropism. Plant shoots upward towards the sun can be called positive phototropism or negative gravitropism because the shoot was programmed to grow towards the light. Gravitropism is divided into three different steps: perception of the gravity vector, transduction of the signal and bending of the responding organ in the appropriate direction. Gravity perception depends on the downward movement of amyloplasts, which is a specialized plastid filled with heavy starch granules. Bending of organs is accomplished via the plant growth hormone named Auxin. Auxins are responsible for cell elongation in the stem but inhibit the root. Thus, increasing auxin levels at the downside of a reoriented shoot provides an upward bending. While accumulation at the bottom half of the root results in downward bending (Massa & Gilroy, 2003).

Phototropism is the orientation of a plant in response to any source of light, either towards the source of light (positive phototropism) or away from it (negative phototropism). The main source of light on Earth is the Sun. Mostly the directions of phototropism and gravitropism are opposite to each other; when this happens, the plant has normal growth, leading to that gravitropism and phototropism are cooperative and interacting processes (Khan Academy, 2022).

2.3.1 Other Tropisms that may be Implicated in Response to Altered Gravity Levels

While investigating gravi-response and response to altered gravity levels, other tropisms, such as thigmotropism, must also be considered. The root cap, which uses the thigmotropic response as an obstacle avoidance technique during soil growth (Massa & Gilroy, 2003), is a plant organ that is highly sensitive to touch. The thigmotropism and gravitropism signals were very similar. Ca^{2+} signaling and auxin redistribution are involved in both tropisms (Legue et al., 1997). Waving and skewing phenotypes in the root are hypothesized to be linked to the interaction between thigmo- and gravitropisms. Skewing has been found in plants cultivated in space, implying that skewing can happen without gravitropism (Califar et al., 2020).

Other tropisms, such as hydrotropism, influence plants to develop in the space environment. Gravitropism and hydrotropism interact in such a way that when hydrotropism is triggered (Kiss, 2007), gravitropism is suppressed, and vice versa (Morohashi et al., 2017). Furthermore, ROS, which increase gravitropism, suppresses hydrotropism. This could mean that roots in microgravity are more sensitive to water gradients than those on Earth, as in an experiment conducted with cucumber seedlings. Furthermore, the distinct behavior of water in the absence of gravity, as well as the manner in which it is provided to plants in various experimental hardware, could have an impact on root growth. Indeed, in spaceflight, this unusual behavior of fluids could be the cause of hypoxia (Choi et al., 2019), which has been found in numerous space tests.

2.4 Effects of Microgravity on Plants

2.4.1 Disrupted Meristematic Competence

Previous space tests have demonstrated that space flight has a negative effect on plant development. In the root experiment (Matía et al., 2005), 4-day-old etiolated *Arabidopsis thaliana* seedlings had disturbed meristematic competence in the root meristem, with increased cell proliferation and decreased cell growth. Meristematic tissue serves as a source of new cells for plant growth and development. For normal plant development (Mizukami, 2001), cell growth and proliferation must be coordinated precisely. Proliferation and growth cycles ensure proper meristematic activity and organ growth because meristematic cells divide when they reach a particular size. Meristematic

competency is impaired when this coordination is broken. This effect of microgravity on meristematic competence was further validated in diamagnetic levitation tests in a simulated microgravity experiment on an RPM as well as in diamagnetic levitation tests (A. I. Manzano et al., 2013).

2.4.2 Cell Cycle

The enhanced cell proliferation observed in microgravity is due to changes in the cell cycle progression, which leads to meristematic competence disturbance. Results in an experiment done on *A. thaliana* showed that in simulated microgravity (Menges & Murray, 2002), cell cycle progression was accelerated compared to the 1 g control (Kamal et al., 2019), which could be attributable to a decrease in G2 phase and a modest increase in G1 (Kamal, Van Loon, et al., 2019). The disruption of the G2/M checkpoint could be the cause of these changes in cell cycle phases. This checkpoint in the cell cycle allows cells of a specific size to divide (D'Ario & Sablowski, 2019). If this control of cell cycle progression is interrupted, smaller cells will initiate division, impairing meristematic competence. In comparison to the 1 g control, a reduction in B1 cyclin expression supports the alteration of the G2/M checkpoint in simulated microgravity. In the G2/M phase (Menges & Murray, 2002). CYCB1 plays a critical role in controlling cell cycle progression.

2.4.3 Transcriptomic Changes Under Microgravity and Spaceflight

Some studies on transcriptome analysis of different seedlings in space have been published in recent years (Zupanska et al., 2013). Proteomic analyses were also performed. These tests were carried out in a variety of locations, with different lines and light conditions, making direct comparisons difficult. However, there are some reactions that are consistent across research. Hypoxia, or an altered oxidative state, is a common response to the space environment (Kwon et al., 2015). Furthermore, the cell wall metabolism was disrupted. Upregulation of Heat Shock Proteins (HSPs) or chaperons is another common change between transcriptome studies (Zupanska et al., 2013). Changes in the cytoskeleton are common in spaceflight. It's been claimed that HSPs and oxidative stress changes are linked to cytoskeleton remodeling in spaceflight. In addition, plastid

genome overexpression and photosynthetic down regulation in microgravity have been shown in many investigations (Ban et al., 2013).

2.5 Constraints of Space Research

Due to the low availability and expensive cost of space research, as well as the intricate logistics involved, it is severely limited. This hampered the repeatability of the results. Furthermore, due to varying experimental settings and, in particular, variances in the hardware used in the studies, it is often difficult to compare results from multiple trials. Therefore, it might be difficult to distinguish between spaceflight effects and hardware artifacts, as studies conducted in the same facility typically have more similarities. In recent years, researchers have attempted to evaluate all known results from various space studies in order to find parallels that may be deemed true spaceflight effects. The Genelab database from NASA (Ray et al., 2019), the Space Omics team from ESA, and the Test of Arabidopsis Space Transcriptome are all part of this program (TOAST). The latter is an interactive tool that includes Genelab's RNA-seq findings and allows for direct comparisons.

Furthermore, the construction and operation of the International Space Station (ISS) have increased facilities for plant space research, resulting in superior physiological development. In this context, the European Modular Cultivation System (EMCS) (Brinckmann, 2005) represented a significant advancement over other commonly used hardware, with features such as seed hydration controlled from the Norwegian User Support and Operations Center (N-USOC), or atmospheric control controlled from the Norwegian User Support and Operations Center (N-USOC) (O₂, CO₂, humidity, ethylene removal and temperature). It also included an integrated centrifuge, which enabled partial gravity study and 1 g control in space. The SG experiment was the first to explore partial gravity effects on higher plants in space, and partial gravity research is quite limited (Valbuena et al., 2018). Other partial gravity effects were studied employing microgravity simulators with customized settings, such as diamagnetic levitation on plant cell cultures (A. I. Manzano et al., 2012) or RPM simulation on cell cultures (Kamal et al., 2018) and seedlings (A. Manzano et al., 2018). Overall, the EMCS served as an outstanding laboratory for biological research. It was,

however, decommissioned in 2018. VEGGIE (Massa et al., 2016) and the Advanced Plant Habitat (APH) are two of the more advanced hardware options accessible on ISS today (Monje et al., 2020). VEGGIE incorporates programmable light-emitting diode (LED) illumination, a fan that circulates the air, and, unlike previous technology, which grew plants on Petri dishes or nitrocellulose membranes, VEGGIE grows plants on a substrate. Adult plants have been successfully grown in space using this gear (Khodadad et al., 2020). The APH, on the other hand, is the most advanced hardware (Monje et al., 2020). Aerial control, light intensity, and spectrum quality modifications, as well as O₂, CO₂, humidity, and temperature management, are all included in this gear. Unfortunately, none of these advanced institutions have a centrifuge that can conduct partial gravity studies.

2.5.1 Simulated Microgravity

Due to the limited number of space experiments and the high cost of doing so, the study of partial and real microgravity on Earth is not feasible. Therefore, the use of gravity simulation tools such as a 2-D clinostat is recommended. The concept of rotating plants around a single axis was first used in the 19th century to prevent the sedimentation of plant statoliths (“V. On the Direction of the Radicle and Germen during the Vegetation of Seeds. By Thomas Andrew Knight, Esq. F. R. S. In a Letter to the Right Hon. Sir Joseph Banks, K. B. P. R. S,” 1806). Despite the long history of this technology being used in scientific studies, there is still no consensus regarding the optimal orientation and speed settings. The correct application of the 2-D clinostat depends on the material and the experiment's size. In previous studies, the use of fast, slow, or variable rotation rates was not justified (Boucheron-Dubuisson et al., 2016).

2.6 RNA Role in Seed Germination

Water uptake is one of the most important factors contributing to seed development. This process is carried out when a part of the embryo is released, which then causes a radical to emerge from the seed coat (Bewley, 1997) . In the case of seed dormancy, this is a temporary failure that can prevent a viable seed from developing. It is an adaptive feature that can help improve the timing of the seed's emergence. The seed's dynamic development is complex and involves a variety of metabolic, cellular, and

physiological events (Bewley, 1997). During the first phase, the solutes are rapidly transported to the surface, which allows for the formation of protein and respiration. During the second phase, the synthesis of new proteins and mRNA begins. The accumulation of mitochondrion helps support the energy requirements of the seed during this phase. During the third phase, the radicle cells divide and elongate. This process is followed by rapid DNA synthesis and replication.

Various environmental factors that can affect seed development include temperature, light, RH, and soil moisture. In addition to these, other factors, such as the duration of the seed's dormancy period and the thickness of its seed coat, can also contribute to its success. Studies have shown that certain phytohormones, such as abscisic acid and brassinosteroids, can influence seed development by regulating various molecular processes involved in seed dormancy. The activity of plant hormones is regulated to ensure that they do not exert conflicting effects on the development of a seed (Martin et al., 2010).

Although the activity of plant hormones is regulated to ensure that they do not exert conflicting effects on the development of seeds, the negative effects of ABA on the germination and maintenance of plants are known to occur. The absence of ABA during seed development can lead to the development of a variety of unusual and potentially harmful seeds (Finkelstein et al., 2008). The role of GA in promoting seed development was similar to that of BR and ethylene in counteracting the negative effects of ABA. Through the discovery of small non-coding ribonucleic RNAs, which have a length of 19 to 24 nucleotides, researchers have gained a deeper understanding of the regulation of the cellular environment. These RNAs have been shown to play various roles in the development and growth of plants and animals. These small ribonucleic RNAs are produced by the activities of two major RNA-dependent protein complexes, which are known as ARGONAUTE and RDR. There are two types of non-coding RNAs that are known to negatively affect the genes that are involved in the regulation of certain cellular processes (Bartel, 2004). These are known as short-interfering and micro-interfering RNAs. At the transcriptional and post-transcriptional levels, the small ribonucleic RNAs can play a role in the remodeling of the chromatin, which is a process that involves the interaction of multiple genes (Huettel et al., 2007). In addition to the

actions of the plant hormones, the function and synthesis of many of the small ribonucleic RNA genes are also regulated by environmental stress and other factors.

2.7 Phytochromes Inhibit Hypocotyl Negative Gravitropism

Red and far-red light spectrum influence plants to have phytochrome-mediated regulation of various light responses, such as seed germination and photomorphogenesis. They also prevent plants from shading. Studies have shown that these phytochromes promote light responses by degrading the PIFs (Phytochrome Interacting Factors). The gravitropic responses of plants are divided into four phases. These include signal generation, gravity sensing, asymmetric elongation, and signal transmissions. To determine the gravitropism of a plant, gravity sensing requires the presence of starch-filled amyloplasts in the root cells (Kim et al., 2011). These are known to be involved in the development of shoot endodermis, which are critical in the regulation of shoot negativity. The short root and the scarecrow mutants do not exhibit shoot negativity. The presence of high levels of amyloplasts starch in the root and shoot can affect the gravitropism of the plant. For instance, the phosphoglucomutase-producing *sex1* mutant has a reduced gravitropic response when compared to the other variants. The presence of several shoot gravitropic variants, which exhibit reduced gravitropism, further highlights the importance of the endodermis, the endodermal vacuole biogenesis, and the gating membrane dynamics in the sensing of gravity (Kim et al., 2011). Disturbed localization and the alteration of amyloplasts movement are known to be the factors that cause the reduction in the gravitropism of these mutants. Once gravity is sensed, the biophysical signal is then converted to a biochemical signal, which is then transmitted to the tissues.

One of the less understood effects of light on plant gravitropism is hypocotyl-negative tropism. This can hinder plant growth by preventing the development of a sufficient response to light. It has been suggested that plants have evolved phytochromes that can inhibit the hypocotyl negative tropism by regulating the light responses. In support of this hypothesis, studies have shown that when negative gravitropism is inhibited, the plants display stronger phototropic responses when exposed to blue light.

2.8 Singlet Oxygen-mediated Programmed Cell Death

Programmed Cell Death (PCD) is a component of plant development that is involved in a wide range of activities. It is also known to be involved in defense reactions during plant-pathogen interactions and in response to abiotic stress. Chloroplasts are the most important sites where ROS is produced. A variety of stress conditions can limit the ability of plants to utilize light energy for photosynthesis (Danon et al., 2006). These conditions can also increase the number of ROS produced in the plastid compartment. Studies have shown that the development of PCD in plants is linked to the impairment of the plant's ability to utilize light energy for photosynthesis. This condition can also lead to the development of a variety of defense reactions. For instance, the production of superoxide and hydrogen peroxide can trigger the plant's hypersensitive response during an incompatibility plant-pathogen interaction. In studies on the effects of singlet oxygen on the development of PCD in plants, the researchers looked into the role of the plastid's ROS production in this defense reaction. It is known that the release of singlet oxygen can trigger the plant's death response. However, this effect is not enough to trigger the development of this condition. Instead, it has to be combined with another blue light reaction. In plants, there are two types of photoreceptors that are known to trigger the development of PCD. One of these is the cryptochrome-1. In the studies, the researchers discovered that cryptochrome-1 could trigger the plant's death response when singlet oxygen is released. When singlet oxygen is released into the plastids of a mutant plant, the changes in the nuclear gene expression can be rapid. In CRY1-mutant plants, the singlet oxygen-dependent upregulation of a small set of genes is suppressed. These genes had previously been associated with various conditions, such as PCD and oxidative stress. However, their expression was not under the control of CRY1 (Danon et al., 2006).

Under light stress, plants generate two types of ROS: photorespiration and photosystem I. The former is designed to maintain the acceptors of the photosynthetic system in a partially oxidized state, which minimizes the risk of photoinhibition. On the other hand, if the plant's capacity to remove excess light energy is not enough to prevent photoinhibition, then enhanced levels of singlet oxygen can be produced by the plant. The conditions that favor the release of hydrogen peroxide and superoxide when

compared to those that stimulate the production of singlet oxygen are known to evoke different stress responses from the plant. Although the two groups of ROS can trigger cell death, singlet oxygen-mediated death differs from that induced by hydrogen peroxide.

2.9 Anaerobic Respiration: The Key to Germinate Under Oxygen Deficiency

The process of seed germination is energy-consuming and requires the use of many resources. To meet the requirements of this process, food reserves must be efficiently utilized. In a normoxic condition (normal oxygen concentration), the free diffusion of oxygen from the air to the seed can be achieved through the aerobic respiration of the food reserve (Narsai et al., 2015). To meet the requirements of the process, the free diffusion of oxygen from the air to the seed can be achieved through the aerobic respiration of the food reserve. However, submergence can prevent the free flow of oxygen from the air to the seed. This is referred to as anoxia, which is a rare phenomenon that rarely occurs during flash floods or in prolonged water stagnation. On the other hand, hypoxia is a real-life issue that can affect the seed germination process. In the case of oxygen deficiency, the energy required for the seed's germination can be obtained from anaerobic respiration (Narsai et al., 2015). This term refers to the process of seed acclimatization under these conditions. It is believed that the inherent capacity of seeds to reproduce under these conditions can be achieved using anaerobic respiration.

2.10 Role of Gibberellic Acid and Other Hormones in Seed Germination

Various classes of plant hormones are responsible for regulating plant growth and development. These hormones function at different sites in plants. They can also be transported to different tissues. The understanding of the various pathways involved in the production and transport of plant hormones has significantly improved over the past two decades. In addition to acting on their own, plant hormones also interact with each other in a complex manner. Seed germination is one of the most important steps in plant growth studies. This process can be performed by analyzing the molecular cues that guide the metabolic activity of plant embryos. Several studies have revealed that the different traits exhibited by the plant's genetic and proteomic properties are related to successful germination. Successful germination of plants is regulated by various

hormones produced by different classes of plant hormones. Some of these include abscisic acid, auxin, and ethylene. In addition to these, other environmental factors, such as the presence of abiotic stresses, can also affect the plant's success. Abiotic stresses are external factors that can affect the development and growth of plants. Some of these include drought, cold, gravity and salinity. These factors can significantly affect the average yield of crops. These three factors can reduce the average crop yield by about 50% (Mahajan & Tuteja, 2005). The level of tolerance of plants towards abiotic stresses is regulated by the complex signaling pathways that are involved in this process. When abiotic stresses are introduced to the plant, they trigger the development of a physiological response that involves the activation of multiple signaling cascades.

Over the past decade, various studies have been conducted on the role of two plant hormones, Abscisic Acid (ABA) and Gibberellic Acid (GA), in plant development and growth. Various roles these hormones play in the regulation of the plant's response to abiotic stresses. Gibberellins play a significant role in plant development and growth. They can control various aspects of the plant life cycle, such as seed germination, the development of stem and leaf extensions, and flowering and fruit development. Due to the varying environmental conditions and developmental stages, the signals and metabolism of the two hormones are affected.

Crosstalk between abiotic stresses and plant growth has been observed in various instances. For instance, in some cases, high levels of ABA in seeds are caused by unfavorable conditions, whereas in other cases, low GA levels are caused by favorable conditions. Seed dormancy is maintained by the ABA, which gradually increases from embryogenesis to maturation (Karssen et al., 1983). The abiotic stress can restrict the growth potential of an embryo by inhibiting the water uptake and cell wall loosening (Schopfer & Plachy, 1984), which are key steps in the start of germination. It also leads to the induction of late-embryogenesis abundant genes (LEA) and growth arrest. These genes are produced by a certain type of protein known as the ABSCISIC ACID INSENSITIVE 5. Some of the LEA genes that are known to contribute to abiotic stress tolerance in plants are ABI5 and ABI3 (Lopez-Molina et al., 2002). These two genes have been shown to suppress the germination of plants through the activation of a variety of pathways. In conditions that are favorable for the development of plants, the

activation of these pathways can lead to the release of the inhibitory effect of ABA. Cold stratification and light exposure can increase the production of bioactive GAs by the transcription factors PIF3 and BME3 (Blue Microphyllar End3). These factors are known to regulate the dormancy and germination of plants. It is also clear that the interactions between the ABA and the GA in seeds can affect the development of plants.

Among the functions of plant hormones are to control and coordinate various plant activities, such as growth and differentiation (Vishal & Kumar, 2018). They can also affect plant dormancy and other activities. Some of the chemicals that plant hormones are known to include abscisic acid, ethylene, and guanine. Soil microbes produce these products in addition to plants. Plant hormones are known to have high-affinity receptors in plants. These molecules are used by prokaryotes and eukaryotic cells to respond to plant hormones. Before a seed can be established, it must first complete a set of stages, such as the availability of food stores.

Food stores are components of a plant ecosystem that can be accessed by seed embryos through the activities of certain enzymes and pathways. For instance, certain proteins known as phytochelatins can help prevent the degradation of certain proteins during seed germination.

Whole-genome analyses have identified a set of genes that are related to development, hormonal activity, and environmental conditions in *Arabidopsis*. Interestingly, the distribution of genes in different regions of a seed is related to the following processes: 1) dormancy and germination, 2) ripening, 3) ABA activities, 4) gibberellins activities, and 5) stresses such as drought (Miransari & Smith, 2014).

The goal of this process is to determine the degree to which certain signals influence the development and maintenance of seed dormancy. For instance, the activity of certain signals can prevent plants from developing seed dormancy.

2.10.1 Seed Germination

Seed germination is carried out through the activation of the embryo by various physiological and morphological changes. Before the seed begins to grow, it absorbs water, which causes its expansion and growth. Once the radicle has grown out of the

outer layers of the seed, the process is complete. Researchers have studied the various processes involved in seed germination to understand how they affect plant hormones produced by Brassicaceae (Hermann et al., 2007). Proteins found in seeds are known to increase during the maturation stage, which occurs when the seeds absorb more nitrogen. The presence of storage proteins in the radicles and shoots of seeds also increases during the maturation stage (Tiedemann et al., 2000). However, this process does not occur simultaneously in any part of the seed. Some of the enzymes that are activated during the activation of these proteins are aminopeptidase and carboxypeptidase. Some of the most important factors that are considered when it comes to the seed's seed dormancy process are the changes in the hormonal and protein levels, including the protein and hormonal alterations and the balance between abscisic acid and gibberellins (Ali-Rachedi et al., 2004).

Various factors that affect seed dormancy, such as related genes, chromatin-related factors, and non-enzymatic processes, are involved in this process. The genes that control dormancy include maturing genes, epigenetic and hormone-regulating genes, and genes that control release from dormancy (Graeber et al., 2012). Using a combination of genetic and non-enzymatic tools can help us to understand the role of each plant hormone in seed germination. During embryogenesis, various changes occur, including the synthesis of mitotic microtubules and DNA. These changes can be used to identify differentiation and cell division during this stage. These processes are parallel to the ability of seeds to tolerate desiccation (De Castro & Hilhorst, 2006).

Embryo body development begins when cell division and differentiation result in the creation of an embryonic organ (Goldberg et al., 1994). This period includes various changes in the size and weight of the embryo, as well as the nutrient storage and the establishment of organs. Following the maturation of the seed, dormancy begins (De Castro & Hilhorst, 2006). Seed maturation can result in various effects, such as decreased seed moisture, dormancy, and increased abscisic acid levels. Researchers have also found that certain compounds, such as nitrogen, can help improve seed germination. These include nitrous oxide, which can stimulate amylase activities (Zheng et al., 2009). By decreasing the production of O_2 and H_2O_2 such products can also alleviate stress by controlling the likely oxidative damage, similar to the effects of antioxidant enzymes,

including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) on plant growth under various stresses (Song et al., 2006).

2.10.2 Seed Dormancy

Seed dormancy is a mechanism that allows seeds to delay germination and wait for better conditions (secondary dormancy). Primary dormancy, however, is caused by the effects of abscisic acid during seed development. Such seeds are unlikely to germinate (Bewley, 1997). Although this hormone does not control seed dormancy, it can activate dormant seeds (Bewley, 1997). Abscisic acid can prevent corn germination by interfering with the cell cycle. Therefore, seeds deficient in Abscisic acid germinate more quickly. Abscisic acid inhibits the cell cycle by activating a residual G1 kinase, which becomes inactive in the absence of abscisic acid. Environmental parameters, such as salinity, acidity, temperature, and light, can influence seed germination by affecting the hormonal balance in seeds (Ali-Rachedi et al., 2004).

Nitrate (NO_3^-) and gibberellins can boost seed germination. NO_3^- can be used as a nitrogen source and a seed germination enhancer. On the other hand, gibberellins promote seed germination by inhibiting ABA activity. It is caused by the activation of catabolizing enzymes and the inhibition of related biosynthesis pathways, both of which reduce ABA levels (Atia et al., 2009). NO_3^- is converted into amino acids and proteins by enzymes such as nitrite reductase, nitrate reductase, and glutamine synthetase.

ABA and gibberellins are required for dormancy initiation and seed germination, respectively (Groot & Karssen, 1992). The gibberellin/ABA balance determines seed germination and the pathways required for seed maturation (White et al., 2000). While ABA regulates seed dormancy and prevents seed germination, gibberellins are required for seed germination (Matilla & Matilla-Vázquez, 2008).

Although plant hormones influence seed dormancy, seed morphological and structural characteristics, such as endosperm, pericarp, and seed coat properties, can also influence seed dormancy. Radicle growth is influenced by both ethylene and gibberellins, with gibberellins being the most important hormone. Although gibberellins are required for mannanase production, which is required for seed germination, ethylene

is not required (X. Wang et al., 2005). However, in gibberellin-deficient mutants, ethylene can act similarly to gibberellins because the seeds can germinate completely (Matilla & Matilla-Vázquez, 2008). The molecular and biological stages of seed germination have been elucidated using proteomic analyses. At various stages of seed germination, the expression of various genes results in the production of proteins required for seed germination and dormancy release. Proteins required for seed germination accumulate after ripening under seed drying conditions, resulting in the release of dormancy (Gallardo et al., 2001).

2.10.3 ABA

While ABA positively affects stomatal activity, seed dormancy, and plant activities under abiotic and biotic stresses such as flooding (Popko et al., 2010), it negatively affects seed germination. Concentrations of 1-10 M, for example, can inhibit seed germination in plants such as *Arabidopsis thaliana* (Müller et al., 2006). Other plant hormones, such as gibberellins, ethylene, cytokinins, and brassinosteroids, as well as their negative interaction with ABA, can, on the other hand, positively regulate seed germination (Hermann et al., 2007). Under stress, ABA can be produced quickly as glucosidase. Furthermore, it has been demonstrated that phosphatase regulators can function as ABA receptors. The movement of ABA across the cellular membrane is affected by pH and the cellular compartment. As a result, it is possible to predict hormone concentrations in different cellular compartments based on the cellular pH and compartment. Different studies have shown that ABA and IAA receptors are located outside the plasma membrane (Miransari & Smith, 2014), indicating that the apoplast may be an important compartment at times.

The role of ABA and its responsive genes in seed germination has been previously demonstrated (Miransari & Smith, 2014). ABA inhibits seed germination by delaying radicle expansion and weakening the endosperm, as well as increasing the expression of transcription factors, which may have an adverse effect on the seed germination process (Graeber et al., 2010).

2.10.4 Ethylene

Ethylene has the most straightforward biochemical structure compared with other plant hormones. However, it can have an impact on a wide range of plant activities (Miransari & Smith, 2014). Similarly to cytokinin, ethylene is perceived by a kinase receptor, which is a two-component protein. In contrast, ethylene receptors are found in the endoplasmic reticulum membrane. Although ethylene can influence various plant activities, such as tissue growth and development and seed germination, it is not clear how ethylene influences seed germination. There are various theories regarding seed germination; some researchers believe that ethylene is produced as a result of seed germination, while others believe that ethylene is required for the process of seed germination. Ethylene can regulate plant responses to various conditions, including stress. Ethylene, for example, can influence plant response to salinity when combined with ABA. Plants produce more ethylene in response to increased salinity, which reduces plant growth and development. The enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) is required for the production of ethylene, which is catalyzed by ACC oxidase. During the time that the seed is stressed, ethylene production is reduced (Mayak et al., 2004). The amount of ethylene increases during the germination of many plant seeds, including wheat, corn, soybeans, and rice, which affects the rate of seed germination (Zapata et al., 2004). ACC can promote seed radicle emergence by increasing ethylene production in the radicle. In terms of the ease with which ethylene can be produced from ACC in the presence of ACC oxidase, ACC has been extensively tested in numerous experiments. It has been demonstrated that ethylene is produced in various plant species during the final stage of seed germination and can contribute to seed germination after dormancy.

2.10.5 Cytokinins

Cytokinins are hormones that regulate a variety of plant activities, such as seed germination. They are present at all stages of germination (Nikolić et al., 2006). They can also influence meristematic cell activity in roots and shoots, as well as leaf senescence. Furthermore, they aid in nodule formation during the establishment of the N₂-fixing symbiosis and other plant-microbe interactions (Murray et al., 2007). The

phosphoribohydrolase enzyme converts the nucleotide into a free base, resulting in the production of active cytokinins (Santner et al., 2009). Cytokinin signaling is very similar to two-component signaling in bacterial species. In this viewpoint, the initiation of phosphorelay by ligand binding is associated with the kinases histidine and asparagine. Perceiving compounds in the nucleus can phosphorylate response proteins, which can either negatively or positively regulate cytokinin signaling. Cytokinins, like auxin, can regulate many genes, including Cytokinin response factors (Santner et al., 2009).

Cytokinins can also improve seed germination by alleviating stress such as salinity, drought, heavy metals, and oxidative stress (Miransari & Smith, 2014). They can be inactivated by cytokinin oxidase/dehydrogenase, which catalyzes the cleavage of their unsaturated bonds. The various functions of cytokinins in different cell types have been attributed to their various activities, such as their effects on seed germination (Miransari & Smith, 2014).

Arabidopsis thaliana contains three histidine kinases that can act as cytokinin receptors (Miransari & Smith, 2014). Cytokinins also have the ability to control seed size, including embryo, endosperm, and seed coat growth. In *Arabidopsis*, endosperm and seed coat growth are followed by embryo growth at a later stage of embryogenesis, which is less related to the final seed size. There are several factors that influence seed number in terms of the number of seeds produced, the most important of which is the available carbon source for seed utilization (Miransari & Smith, 2014).

2.11 Effect/Problems of Microgravity on Plants

In general, living beings perceive microgravity as a stress-inducing environmental factor, although the severity of the physiological changes varies greatly between organisms (Bizzarri et al., 2015). In the generation of a biological response, two effects of the altered or null gravity signal overlap: the direct effect is the modification of physiological processes (e.g., changes in the mechanisms of biochemical reactions); the indirect effect is the physical change of collateral elements or factors that are not intrinsically part of the physiological processes but may have an influence on them. Examples of these side effects include water diffusion in the soil, which can affect root hydration, and convective processes, which can affect gas exchange. When analyzing the

experimental results, it is difficult to distinguish between these two types of effects, although some attempts have been made using simulated microgravity (Herranz et al., 2013). These indirect effects can be mitigated by providing adequate ventilation and watering systems, as is currently done in the Veggie and APH facilities on the ISS (Massa et al., 2016).

Furthermore, because terrestrial organisms have evolved under a constant gravity vector, these effects are difficult to overcome (Bizzarri et al., 2015). Although terrestrial organisms do not appear to be prepared to be confronted with an environment devoid of gravity force, and they do lack genetic equipment specialized in response to this condition, signs of acclimation and adaptation processes have been found in some species as a result of their genetic plasticity, a feature that varies greatly between taxa (Paul et al., 2012). Comprehensive research on these processes is still lacking, and it appears that, similar to the intensity of gravitational stress, different taxonomic categories exhibit varying adaptability.

Gravity, in the case of higher plants, is an environmental factor that has a decisive effect on plant growth through a process called gravitropism, which modulates growth orientation according to the gravity vector, with positive roots gravitropism and negative shoots gravitropism. However, cues for this tropism are absent in microgravity. As a result, gravitropism is replaced by automorphogenesis, a process that produces spontaneous curvatures in roots followed by straight root elongations in random directions (Hoson et al., 2003). This is accompanied by a significant reorganization of phytohormone transport and distribution, primarily affecting auxin but also cytokinin, according to data obtained in real and simulated microgravity (A. I. Manzano et al., 2013). This hormonal perturbation is transduced to the meristem, altering cell proliferation rate and ribosome biogenesis during seedling development, which may have consequences at the level of the plant's developmental pattern, ultimately relying on meristematic activity. Furthermore, one observed effect of microgravity is the induction in the plant of increased sensitivity to other environmental cues, such as moisture or light, which are typically masked by dominant gravitropism (Medina et al., 2021).

Changes in gene expression have been linked to physiological and cellular changes, as revealed by transcriptomic studies from space and ground experiments (Paul et al., 2012). Complementary proteomic studies were also carried out in some cases (Kruse et al., 2020). Aside from real or simulated microgravity, the environmental conditions of these experiments were far from uniform, making direct comparisons difficult. To alleviate this inconvenience, the NASA-led GeneLab initiative has undertaken a significant effort in data sharing and harmonization (Ray et al., 2019). This effort has resulted in the identification of some common responses, which are frequently associated with general mechanisms of stress defense, such as the system of heat shock genes that produce Heat Shock Proteins (HSP), which are molecular chaperones that act to protect and refold proteins in response to cellular damage. Furthermore, the oxidative stress pathways, which involve the production of Reactive Oxygen Species (ROS), the cell wall remodeling system, cytoskeleton alterations, and plastid genes, including those regulating photosynthesis, were included in the core set of functions that appeared to be affected by the spaceflight environment (Kwon et al., 2015). Specific genes involved in plant responses to microgravity or the spaceflight environment have not been discovered in any of these transcriptomic studies.

2.12 Effect of Radiation on Plants

According to the Health Physics Society, radiation has a positive effect on plant growth at low levels and a negative effect at high levels. Plants require non-ionizing radiation, such as sunlight, for photosynthesis. Although solar radiation is essential for plant survival, some other types of non-ionizing and ionizing radiation are harmful to plants. Plant growth and sprouting are affected by ultraviolet radiation, and the amount of damage is proportional to the amount of radiation received. Because of radiation exposure, soil can compact and lose nutrients necessary for plant growth. Experiments in laboratories using filtered lamps to deliver ultraviolet radiation to plants demonstrated that higher doses of radiation were highly damaging. Radiation disrupts stomatal resistance. Stomata are small air holes within plant leaves that regulate water levels. When there is excessive evaporation as a result of intense radiation, the stomata close to conserve water. The plant's growth is slowed when the stomata are unable to open for an extended period of time. Prolonged radiation exposure can destroy the plant by

completely damaging the stomata. Plant cells contain genetic material that allows plants to reproduce. If the cell is severely damaged by radiation, reproduction is hampered. The mutation is more likely when UV radiation destroys cells. Affected plants are frequently small and weak, with irregular leaf patterns. Plants are killed by intense radiation in various ways. The reactivity and sensitivity of trees and shrubs to radioactive substances vary. This variation is caused primarily by differences in size and chromosome number. Sparrow reported that plants with fewer chromosomes are more vulnerable to radiation attack than those with an excess of small chromosomes. Some plants, such as pine trees, died right away. Near nuclear power plants, radiation pollution is relatively high, and many radionuclides, particularly caesium 137, iodine 131, strontium 90, and carbon-14, accumulate in plant tissues growing within this region. Plants absorb the most light near 280 nm, making plant proteins more likely to be exposed to ultraviolet radiation. UV radiation causes a 20%–50% reduction in chlorophyll content and harmful mutations in plants. According to an Australian National University study, UV-B radiations reduce the effectiveness of plant photosynthesis by up to 70%. Because of the intense UV radiation, more surface water evaporates through the stomata of the leaves, resulting in a decrease in soil moisture content.

In this context, plant growth and morphogenesis will be greatly modified under space conditions. Both tropic movements are interestingly controlled by native plant growth hormones. Therefore, the study with the date palm seeds maintained at the ISS over a period, compared with the seeds maintained at ground level, will be of considerable interest in assessing the growth responses at the molecular level. These studies will provide an insight into how the seeds recoup in microgravity to conditions with higher gravitational pull.

2.13 Objectives

The main objective of this project is to investigate the molecular responses of date palm seeds to microgravity and the accompanied challenges faced in space.

Following are the objectives proposed: -

- Evaluating the germination of the date seeds exposed to microgravity.
- Transcriptomics analysis of the microgravity and gravity seeds.

- Functional profiling of the Differential Gene Expression (DGE).

Chapter 3: Materials and Methods

3.1 Seed Material

Date palm seeds were used in this study to better understand metabolic changes caused by long-term exposure to microgravity at the International Space Station. The research methodology entailed analyzing seeds kept in two different environments: microgravity and gravity. The freshly collected seeds were cleaned for five minutes in a bleach and detergent solution before being briskly stirred at least ten times in distilled water. A total of 90 seeds of various varieties which are Lulu, Majdool, Mesalli, Navadoor, Nabtat and Sukkari were sent to ISS, with 10 -15 seeds of each variety: Lulu, Navadoor, Medjool, Sukkari, and Mesalli. It was kept in space for six months before being brought back to Earth for analysis. Copies of the same varieties were used to maintain the gravity situation in order to make an accurate comparison.

Seeds were air-lifted from Abu Dhabi, United Arab Emirates (UAE) to the National Aeronautics and Space Administration (NASA), USA, in sealed aluminum foil bags (company appointed by NASA). The storage was kept at room temperature (24°C), but because no temperature data logger was used, we were unable to detect any occasional fluctuations outside this temperature range. On September 25, 2019, space seeds were loaded onto a Soyuz MS-15 launch vehicle bound to the International Space Station. Space seeds were stored inside the ISS for six months (mean temperature of 22 to 23°C with occasional fluctuations between 18 and 32°C), and their location within the space station changed on occasion. After six months on the International Space Station, the seeds were returned to gravity in a return launch and then airlifted to Abu Dhabi, UAE from Houston, USA. Throughout the space journey, the sealed foil bag was supposed to maintain optimal humidity, air composition, and pressure. When the seeds were returned, they were packed in dry ice and transported to the UAE University biotechnology lab, where they were kept at (-) 80°C until they were used in the germination experiments. The seeds kept in gravity were also kept in the same conditions until they were used in the experiments.

3.2 Seed Germination

Three seeds from six different varieties, as well as their respective controls (microgravity and control seeds), were slowly thawed, followed by scarification to speed up germination by dipping the seeds in 20% sulphuric acid for 20 minutes and gently shaking them. To remove the H₂SO₄, the seeds were washed at least 5 times in distilled water. After scarification, the seeds were sterilized in 20% bleach for 20 minutes before being gently washed five times in distilled water. The seeds were planted in magenta boxes containing half Morishige and Skoog medium with 2% phytigel and a pH of 5.8 and stored at 25°C in the dark. Two weeks after inoculation, the control/gravity seeds began to germinate. Seeds kept in microgravity on the International Space Station did not germinate.

3.3 Germination of Space-maintained Seeds in Comparison with Gravity Seeds Using GA and Cytokinin

After three months of germination experiment, all control/gravity seeds germinated, but none of the microgravity seeds germinated. Hormonal treatment with 4.6 µM gibberellic acid (GA3) and 1 mg/L cytokinin in half MS medium with 2% phytigel was given to improve germination. Along with gravity seeds, microgravity seeds were planted. The seeds were kept in the dark for 10 days before the gravity seeds germinated.

3.4 Transcriptome Studies

According to the plan, the transcriptome analysis needed to be performed after the seed germination from gravity and microgravity. As the seeds did not germinate, we initiated transcriptome studies on the seeds. However, date palm seeds are hard and contain large polysaccharide fractions, which can physically trap RNA and be entrained during centrifugation and discarded during phase separation, thus leading to low yield. In this context, we have attempted two procedures to extract RNA from seeds. To maintain the same conditions, the seeds were not soaked prior to RNA extraction. The seeds were powdered in liquid nitrogen using a mortar and pestle.

3.4.1 RNA Extraction

The powdered seeds were processed for RNA extraction. RNA extraction was performed using the RNeasy Plant Mini Kit (Qiagen, USA) following the manufacturer's instructions. The quality of RNA extraction was low; therefore, we also tried RNA extraction using a manual method. Powdered date palm seeds were used for RNA extraction according to the method described by (Chang et al., 1993). After RNA extraction, the samples were sent to Agrigenome Labs, India for transcriptome sequencing. Transcriptome sequencing was carried out using Illumina HiSEQ2500 with three biological replicates.

3.5 Bioinformatics Analysis Pipeline

The following bioinformatics steps (Figure 1) are performed for analysis of the data.

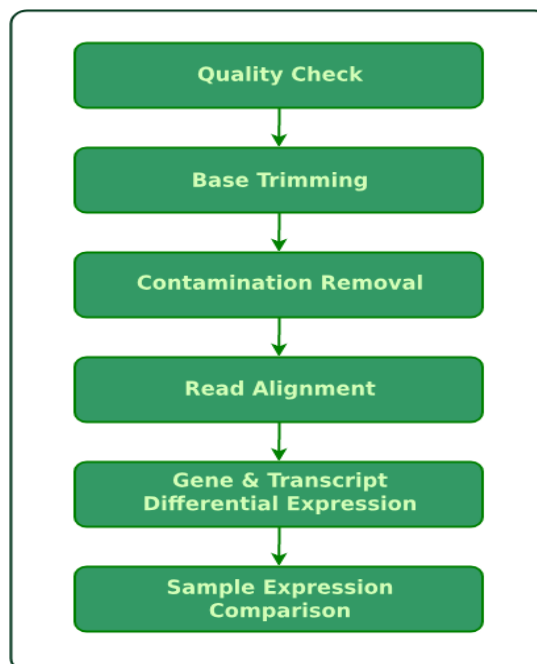


Figure 1: Bioinformatics Analysis Pipeline

3.5.1 Read Quality Check

The following parameters in FASTQ files will be checked for the quality

Base quality score distribution, Average base content per read and Guanine-Cytosine GC distribution in the reads.

3.5.2 Preprocessing and rRNA Removal

In the preprocessing step of the raw reads, adapter sequences and low-quality bases were trimmed using Adapter Removal-v2 (Schubert et al., 2014) (version 2.3.1). Ribosomal RNA sequences were removed from the preprocessed reads by aligning the reads with a ribosomal RNA sequence database consisting of sequences from silva (Quast et al., 2012) and rfam (Kalvari et al., 2021). Alignment with the ribosomal RNA database was performed using gem-mapper (Marco-Sola et al., 2012) (version 3.6.1) and subsequent workflow using samtools (Danecek et al., 2021)(version 1.15.1), bbmap (Bushnell, 2014) (version 38.57) tools, and in-house scripts.

3.5.3 Read Alignment

The preprocessed and rRNA-removed reads were aligned to the Date Palm Genome downloaded from the NCBI database (Hazzouri et al., 2019). Alignment was performed using STAR software (Dobin et al., 2013) (version 2.7.6a).

3.5.4 Differential Expression Analysis

After aligning the reads with date reference genome, differential expression analysis is performed using cuffdiff program of cufflinks package (Trapnell et al., 2012) (version 2.2.1).

Chapter 4: Results

4.1 Germination Experiment on Microgravity Seeds and Gravity Seeds

The germination experiment carried out using space (microgravity) seeds and control (gravity) seeds showed no signs of germination in the microgravity seeds. Date palm seeds of all varieties were maintained under gravity, which served as a control, germinated on the 7th day. The germination rate of control seeds was 100%; however, none of the gravity seeds germinated during the same period. Exogenous GA pulsing of microgravity seeds to force germination also resulted in a lack of germination.

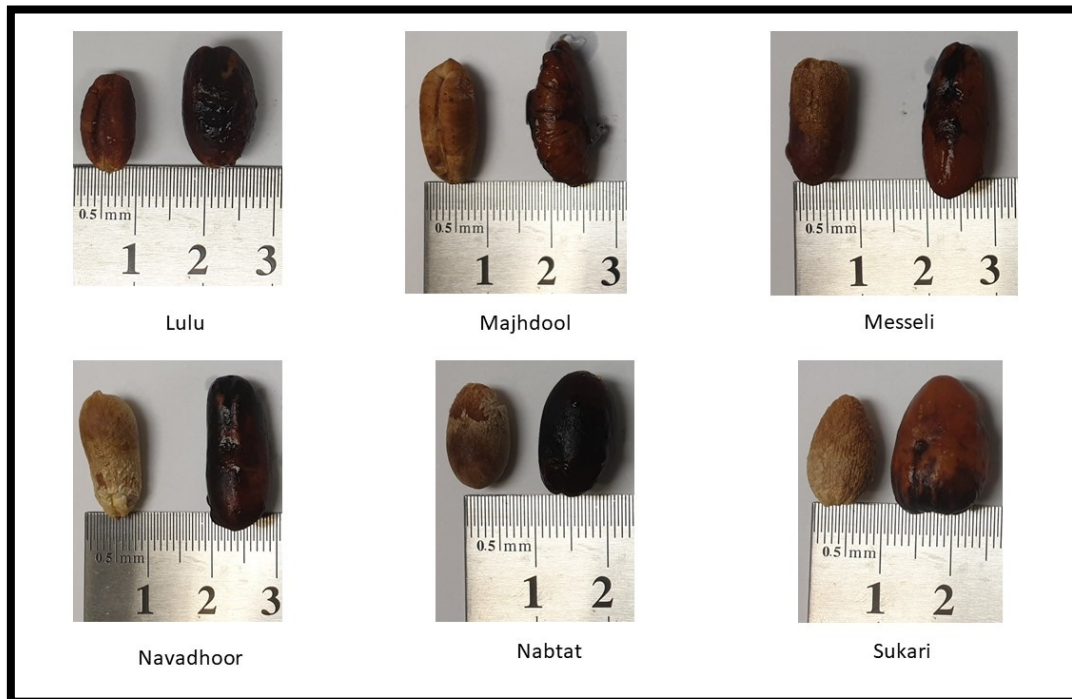


Figure 2: Signs of imbibition of water after one month of initiation of the germination experiment in comparison with dry seeds stored at (-) 80°C.

Signs of water uptake and imbibition were visible in the space where the seeds returned from microgravity; however, there was no radicle emergence as shown in Figure 2. The germination experiment was continued for a month, and the germinated control seeds were stored at (-) 80°C.

4.2 RNA Extraction

RNA extraction was performed on date palm seeds in replicates to perform transcriptome sequencing. From the kit method, RNA extracted was of low quality; thus, we proceeded with the traditional manual method where we were able to extract good-quality RNA with a good RNA integrity number (RIN) value. Overall, RNA integrity was the major factor affecting the quality of the sequencing data. Therefore, RNA-Seq libraries require high-quality RNA. Prior to library construction, it is necessary to assess the RNA integrity number (RIN), which has become a widely accepted standard for quality measurement and has proven to be more accurate than UV spectrophotometric measurements and pure ribosomal RNA ratios (Schroeder et al., 2006; Sigurgeirsson et al., 2014). The QC analysis and RNA concentration using the CTAB Trizol method are shown in Figure 3. Similar results were obtained for the Lulu and Majdhoor varieties.

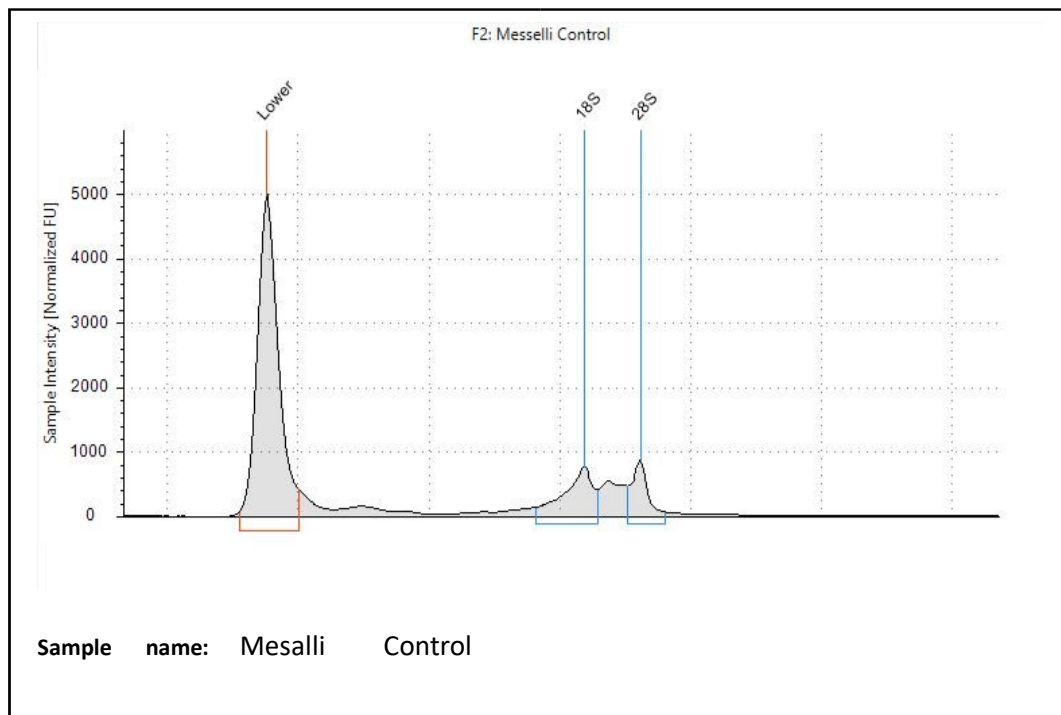


Figure 3: QC analysis of Mesalli sample using the CTAB trizol method. 18s and 23s subunits of RNA were clearly visible in this method with good RNA concentration.

4.3 Data Summary

The FASTQ file summary of the samples is shown in Table 1.

Table 1: Raw FASTQ summary of date palm seeds after sequencing

Sample Name	Number of raw reads	Number of bases (Mb)	Mean-Phred score	Guanine-Cytosine (%)	% Data \geq Q30
Lulu control	21,662,656	3,249.40	39.43	51.94	95.92
Lulu space	24,198,213	3,629.73	39.44	51.44	95.94
Majdool control	23,586,265	3,537.94	39.35	51.88	95.60
Majdool space	23,392,739	3,508.91	38.93	53.67	94.02
Mesalli control	18,475,123	2,771.27	39.35	53.25	95.62
Mesalli space	12,431,522	1,864.73	38.74	48.79	93.36

Transcriptome analysis revealed that all date palm seeds yielded Q30 values greater than 90%. The GC content of the date palm seed samples ranged from 48 to 51%. Higher reads were observed in the Lulu space samples, whereas lower reads were observed in the Mesalli space.

4.4 Alignment with Date Palm Genome

The preprocessed and rRNA-removed reads were used for reference-based pairwise alignment with the Date Palm NCBI GCF_009389715.1 reference genome. The overall alignment summary is shown in Table 2 next.

Table 2: Read alignment summary of date palm seeds after RNA seq.

Sample Name	Total Read Count	Read Count after rRNA removal	Quality Control Pass %	Aligned Read Count	Aligned %	Unaligned %
Lulu control	43,325,312	34,111,670	78.73	27,788,394	81.46	18.54
Lulu space	48,396,426	45,306,428	93.62	40,387,256	89.14	10.86
Majdool control	47,172,530	45,735,196	96.95	41,699,268	91.18	8.82
Majdool space	46,785,478	21,284,826	45.49	13,880,739	65.21	34.79
Mesalli control	36,950,246	35,838,124	96.99	32,371,556	90.33	9.67
Mesalli space	24,863,044	22,812,472	91.75	20,160,080	88.37	11.63

4.5 Differential Gene Expression Analysis

Differentially expressed genes were identified in each variety, with 108 upregulated and 79 downregulated genes in the Lulu variety at a P-value of 0.01 cutoff. In addition, 163 upregulated and 130 downregulated genes were identified in the same variety when the cutoff was 0.05. In the same way, 136 upregulated and 352 downregulated genes were observed in Majdool samples at a P-value of 0.01 cutoff, whereas at 0.05 cutoff, upregulated genes were 229 and downregulated genes were 554. In the case of Mesalli space and control samples, upregulated genes were 127 and downregulated genes were 205 at 0.01 cutoff and 228 upregulated and 402 downregulated genes at 0.05 cutoff. Differential expression analysis for sample comparisons was performed using the cuff diff program in the Cufflinks package (Tables 3 and 4).

Table 3: Differentially expressed genes (p-value \leq 0.01)

Comparison (A vs B)	Up-regulated genes	Down-regulated genes
Lulu Control vs Lulu Space	108	79
Majdool Control vs Majdool Space	136	352
Mesalli Control vs Mesalli Space	127	205

Table 4: Differentially expressed genes (p-value ≤ 0.05)

Comparison (A vs B)	Up-regulated genes	Down-regulated genes
Lulu Control vs Lulu Space	163	130
Majdool Control vs Majdool Space	229	554
Mesalli Control C vs Mesalli Space	228	402

4.6 Differential Expression FPKM Plot

The differential expression FPKM plot of the sample comparison is given in Figure 4.

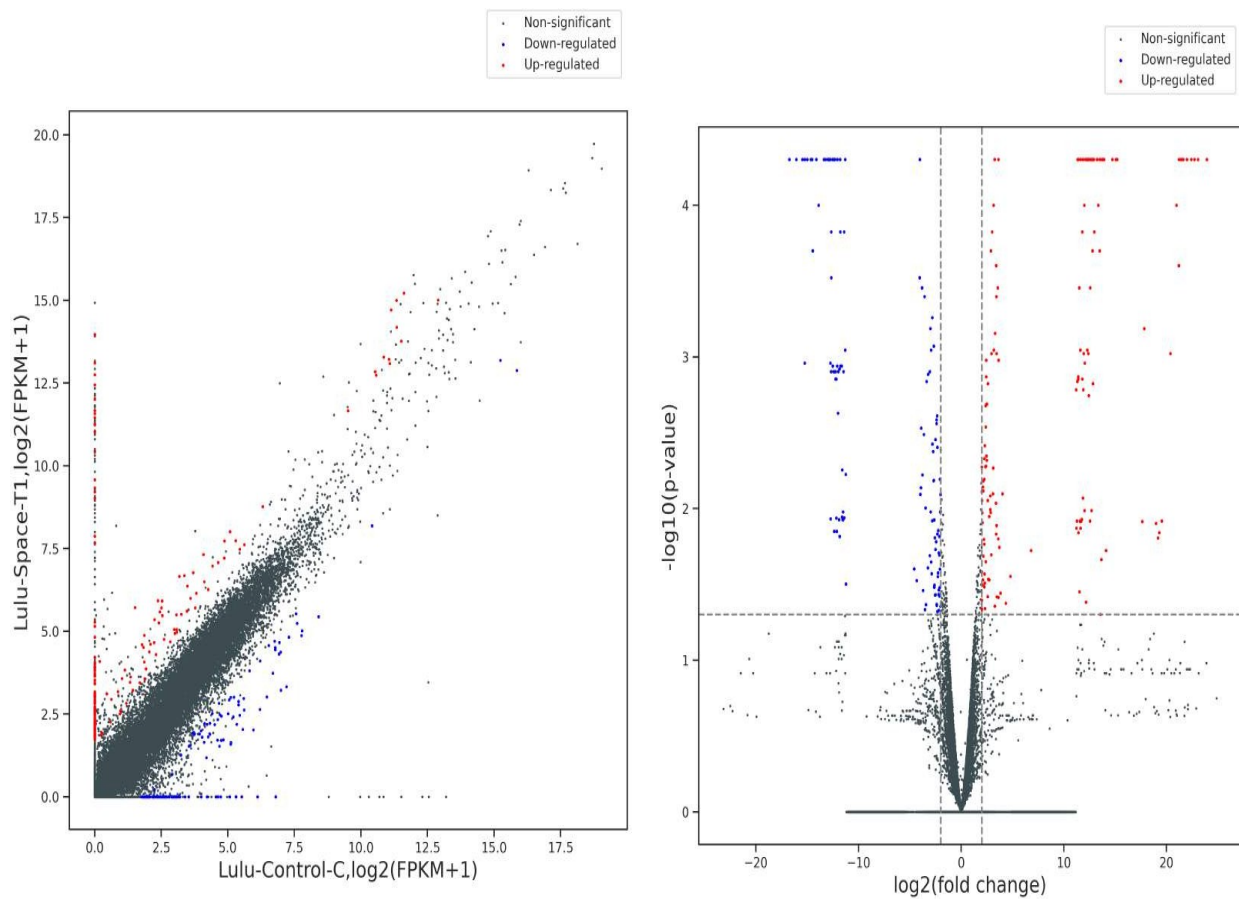


Figure 4: FPKM plot and volcano plot of the Lulu control vs. Lulu space differential expression.

The X and Y axis denote the log 2-fold change and $-\log_{10}(\text{p-value})$, respectively. The red dots represent upregulated genes, blue represents downregulated and black represents the non-significant genes as shown in Figure 4.

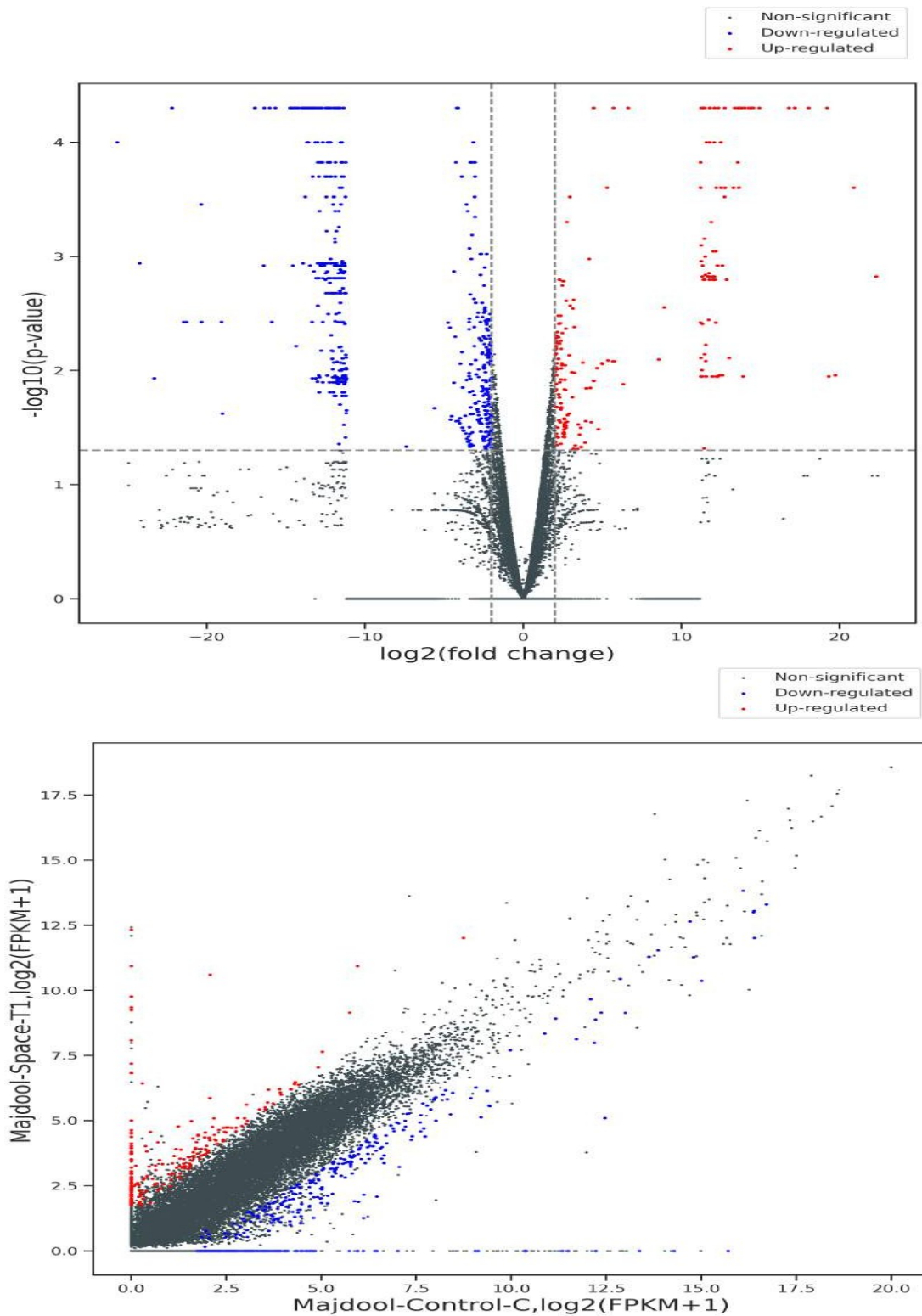


Figure 5: FPKM plot and volcano plot of Majdool control C vs. Majdool space differential expression.

As shown in Figure 5, the X axis and Y axis denote the log 2-fold change and $-\log_{10}(\text{p-value})$, respectively. The red dots represent upregulated genes, blue represents downregulated and black represents the non-significant genes.

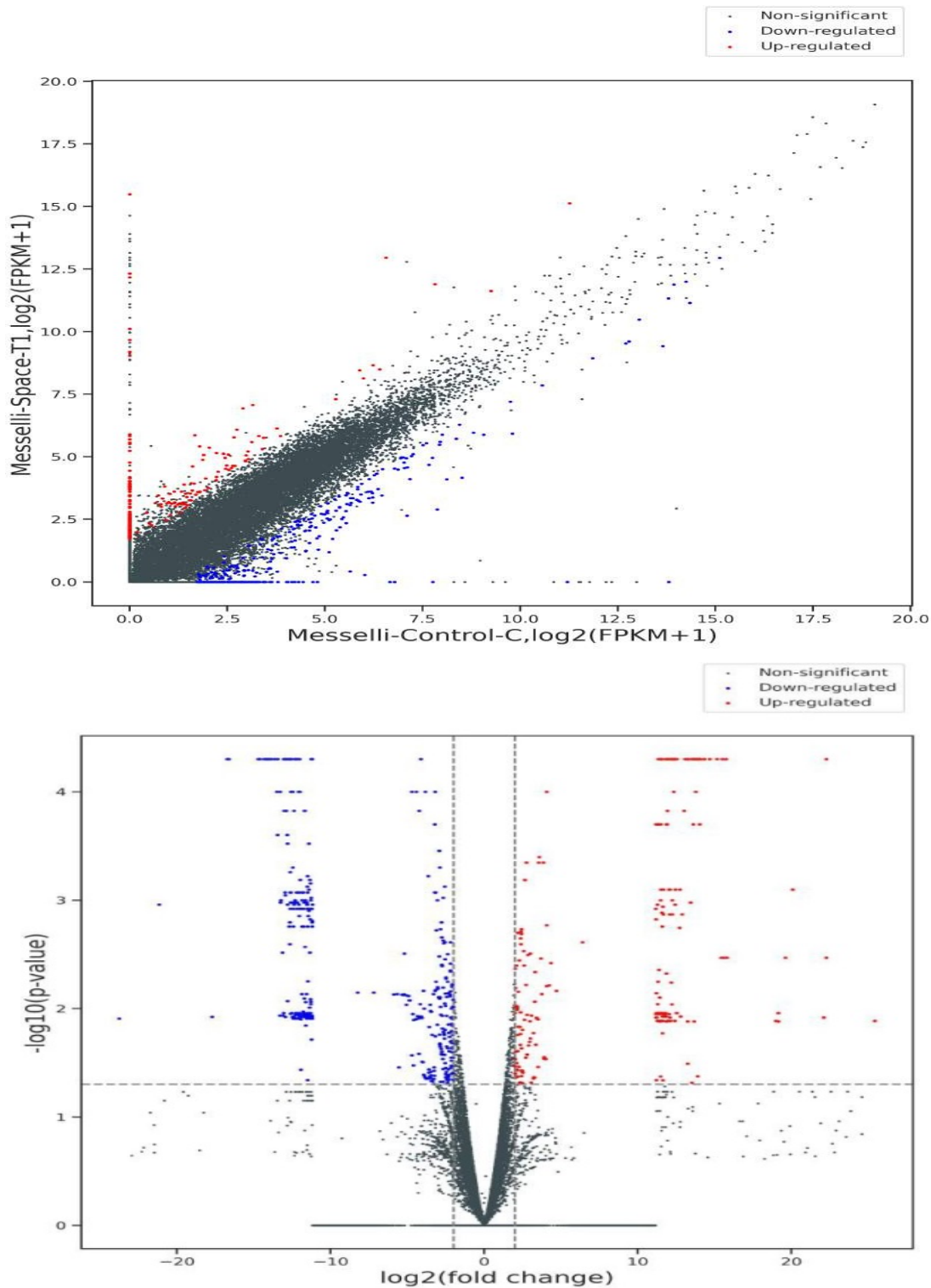


Figure 6: FPKM plot and volcano plot of Mesalli Control vs Mesalli Space differential expression.

As shown in Figure 6, the X-and Y axis denote the log 2-fold change and $-\log_{10}$ (p-value), respectively. The red dots represent upregulated genes, blue represents downregulated and black represents the non-significant genes.

4.7 Functional Profiling of Differentially Expressed Genes

Functional profiling of the differentially expressed genes identified in the Lulu, Majdool, and Mesalli analysis was carried out using the g: Profiler program (Raudvere et al., 2019). Since date palm (*Phoenix dactylifera*) is not a listed species in g: Profiler, the corresponding protein matches in the available *Zea mays* species were identified and used instead. In all three sample comparisons, the differentially expressed genes were identified with a p-value cutoff of 0.05 and log2-foldchange cut of 2. The corresponding maize protein ids were identified using the Blastx option of the Diamond tool (Buchfink et al., 2015).

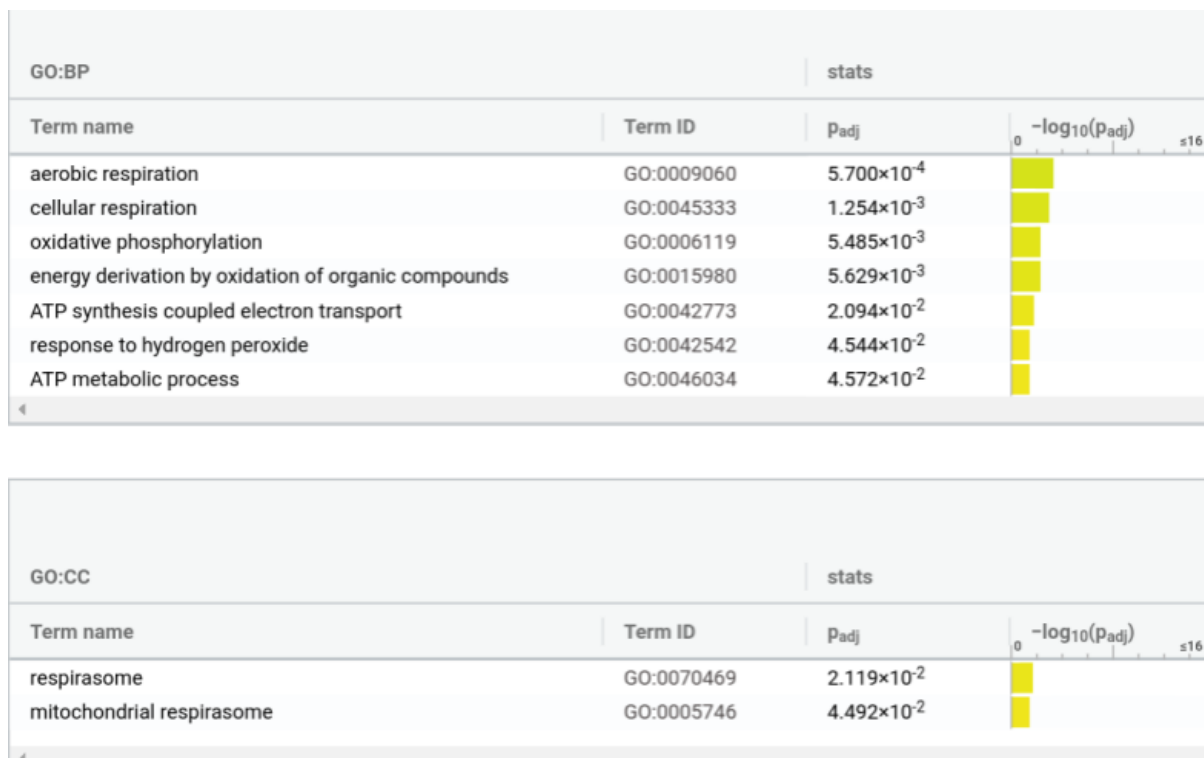


Figure 7: Summary of the results obtained with a p-value threshold of 0.05 between the G profiler for the Lulu control and lulu space. In the Lulu sample analysis, the GO:0009060 term, corresponding to aerobic respiration, was observed among the most significant results.

In the Lulu control vs. Lulu space analysis, 293 differentially were identified. Maize matches were obtained for 217 genes using the diamond program. The matched maize ids were used as input for functional profiling with Gprofiler software. A summary of the results obtained with a p-value threshold of 0.05 is shown in Figure 7. In the Lulu sample analysis, the GO:0009060 term, corresponding to aerobic respiration, was observed among the most significant results.

In the Majdool control vs. Majdool space analysis, 783 differentially expressed genes were identified. Maize matches were obtained for 607 genes using the diamond program. Matched maize ids were used as inputs for functional profiling with a profiler. A summary of the results obtained with a p-value threshold of 0.05 is shown in Figure 8.

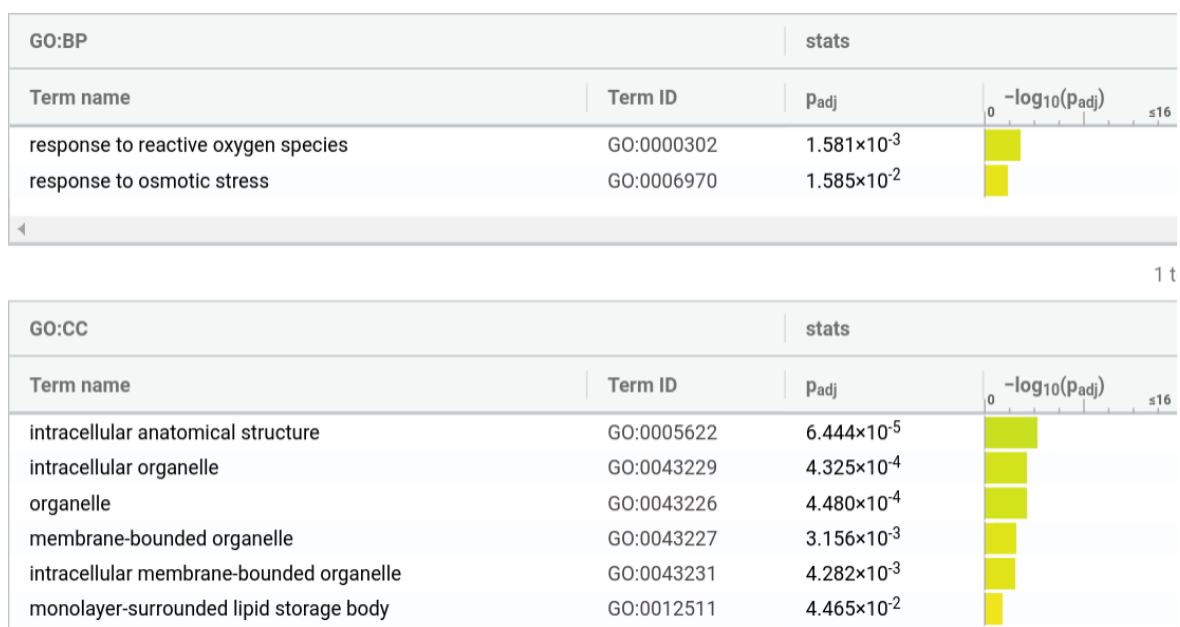


Figure 8: Summary of the results obtained with a p-value threshold of 0.05 between the G profiler for Majdool control and Majdool space. The intracellular anatomical structure (GO:0005622) was among the top log values.

In the Mesalli control vs Mesalli space analysis, 630 differentially expressed genes were identified. Maize matches were obtained for 498 genes using the diamond program. Matched maize ids were used as inputs for functional profiling with a profiler. A summary of the results obtained with a p-value threshold of 0.05 is shown in Figure 9.

GO:MF			stats
Term name	Term ID	P _{adj}	$-\log_{10}(P_{adj})$
structural molecule activity	GO:0005198	6.936×10^{-4}	
structural constituent of ribosome	GO:0003735	7.612×10^{-4}	

GO:BP			stats
Term name	Term ID	P _{adj}	$-\log_{10}(P_{adj})$
peptide metabolic process	GO:0006518	1.227×10^{-2}	
translation	GO:0006412	2.942×10^{-2}	
peptide biosynthetic process	GO:0043043	3.939×10^{-2}	

GO:CC			stats
Term name	Term ID	P _{adj}	$-\log_{10}(P_{adj})$
cytosolic ribosome	GO:0022626	9.310×10^{-5}	
ribosomal subunit	GO:0044391	4.904×10^{-4}	
ribosome	GO:0005840	6.995×10^{-4}	
cytosolic small ribosomal subunit	GO:0022627	4.917×10^{-3}	
respirasome	GO:0070469	2.592×10^{-2}	
respiratory chain complex	GO:0098803	2.692×10^{-2}	
small ribosomal subunit	GO:0015935	3.583×10^{-2}	

Figure 9: Summary of the results obtained with a p-value threshold of 0.05 between the G profiler for Mesalli control vs. Majdool space. Cytosolic ribosomes (GO:0022626) were among the most significant results.

Chapter 5: Discussion

Space exploration is one of the most fascinating human activities. It is becoming increasingly evident that plants are an essential companion of humans in space exploration as a privileged source of high-quality nutrients, oxygen, and waste recycling systems; that is, as a key component of bioregenerative life support systems. Thus, the search for the most efficient way of cultivating plants in space, including knowledge of the mechanisms of adaptation of plants to the space environment and the careful selection of plant species, varieties, and genetic lines, are currently major challenges in plant space research (Medina et al., 2021). The most difficult parameter to overcome in the space environment is the altered gravity level because all other parameters, such as oxygen and nutrient levels, humidity, temperature, and light conditions, can be adjusted. Only cosmic radiation represents a challenge, comparable to gravity, because of the lack of 100% effective protective shields. On the other hand, plants, like every organism on Earth, have evolved under a constant gravity level, and its modification or absence may be a difficult challenge to overcome for an organism. Over the past few decades, human activities in space exploration have continued to achieve breakthroughs. It is possible for humans to have long-term habitation of space and extraterrestrial colonization. Therefore, the establishment of controlled ecological life support systems that can provide basic life safeguards in space has become increasingly urgent (Medina et al., 2021). Higher plants, which are integral components of this system, can produce food and contribute to air revitalization and water purification. Therefore, it is important to study the spatial growth and development of higher plants. Understanding the mechanisms by which cells perceive and respond to gravity is of fundamental relevance in space biology. Land plants have evolved mechanisms that can orient their growth with respect to the Earth's gravitational field. The question of how plants respond to a decrease in gravitational field strength has stimulated research on their responses to gravity. Such research is necessary to provide reliable life support systems for future space missions.

In collaboration with the UAE Space Agency, we sent 90 date palm seeds of three varieties to space which are Lulu, Majdool and Mesalli , which is the basis of the UAE's

initiative to send date palms to Mars. Pilot research has been accomplished by sending date palm varieties to the International Space Station (ISS) as part of the wide horizon of activities to follow with respect to space research. Thus, it was hypothesized that after being maintained in outer space over a period of time, these seeds might undergo morphogenetic changes that can affect their growth and development. These studies will be a basic step toward understanding the implications of a hypothetical transfer from Mars to Earth in the future.

A comparative study on germination carried out using microgravity and gravity seeds showed that microgravity seeds showed no signs of germination. Date palm seeds of all varieties were maintained under gravity, which served as a control, germinated on the 7th day. The germination rate of control seeds was 100%; however, none of the microgravity seeds germinated during the same period. Exogenous GA pulsing of microgravity seeds to force germination also resulted in a lack of germination. Seed aging, deterioration, and senescence are defined as loss of quality over time. The most noticeable signs of seed aging at present are the increasing percentage of individuals in seed lots that do not germinate (viability reduction) or germinate slowly (vigor reduction) over time. Seed aging is a complex biological process that involves several interrelated molecular, biochemical, physiological, and metabolic processes. This makes the phenotype of seed longevity difficult to measure or predict, especially because it is difficult to assess, as the time before detectable changes occur is unknown. Walters et al. showed that in a homogeneous seed lot, there is usually a threshold at which individuals suddenly lose their viability. In recent years, much effort has been devoted to research on seed aging processes; however, the causes of seed senescence have not been fully elucidated. Furthermore, studies on the transcriptome of seeds stored in a dry state and that suffer a loss of viability during storage are very limited.

Transcriptome is a complete set of cell transcripts that are characteristic of developmental or physiological states. Understanding transcriptome activity is essential for interpreting the functional elements of the genome, revealing the molecular components in cells and tissues, and understanding developmental processes. The development of new high-throughput sequencing methods has provided an alternative for mapping and quantifying transcripts (Z. Wang et al., 2009). RNA sequencing (RNA-

Seq) using the transcript profiling approach through deep sequencing and research has changed the view of the complexity of transcripts. It provides a precise measurement of the transcript expression levels and their isoforms. The key aim of transcriptomic research is to catalogue different types of RNA molecules (mRNAs, sRNAs, and non-coding RNAs) to determine the transcriptional structure of genes in relation to their starting point, splicing pattern, and other post-transcriptional modifications to quantify changes in the expression levels of each transcript during development and under different environmental conditions. Using the kit methods, the RNA concentration was very low; however, according to the CTAB and Trizol method, we obtained 158 ng/ μ l RNA with good purity of 1.79, along with a better RIN value of 7.2.

The gravity seeds showed differential expression compared to the microgravity seeds at the transcriptomic level. Since date palm (*Phoenix dactylifera*) is not a listed species in g: Profiler, the corresponding protein matches in the available *Zea mays* species were identified and used instead. Gene ontology enrichment analysis was performed to predict the role of transcripts in the biological, cellular, and molecular processes of cells. In all three sample groups, more than 50% of the differentially expressed genes were involved in various GO terms. In all three sample comparisons, the differentially expressed genes were identified with a p-value cutoff of 0.05 and log₂-foldchange cut of 2. In the Lulu sample analysis, aerobic respiration was observed among the top significant results, whereas the Majdool intracellular anatomical structure (GO:0005622) was observed with higher differential expression, whereas cytosolic ribosome (GO:0022626) was observed in Mesalli varieties.

Seed germination is a highly energy-demanding process, and the energy required is obtained by catabolizing the reserved food contained in the seed itself. At normal oxygen concentrations, where there is no hindrance in the free diffusion of oxygen from air to the germinating seed, aerobic respiration of the stored food reserve is the main mode of deriving the required energy. Nevertheless, during such oxygen-deficient conditions (in whatever form it might be), the energy required for germination is obtained from an alternate resort, that is, by anaerobic respiration. In this context, we observed that the space-travelled seeds or the microgravity seeds of date palm were imbibed with water, but germination did not occur irrespective of treatment with

hormones. Seed imbibition triggers several biochemical and cellular processes associated with germination. As water is taken up by the dry seed, it triggers the transition from seed to germination, which completes with the emergence of embryonic radicle tissues from the seed coat. The germination of cereals is controlled by complex signaling networks, including both internal and external cues. Phytohormones such as ABA and GA act as hubs that connect internal and external signals, antagonistically controlling germination, whereas other phytohormones, carbohydrates, ROS, NO, microRNAs, light, and temperature also affect germination at the transcriptional, translational, and post-translational levels. This observation is in accordance with the studies conducted by Gruwel et al. (2001) on barley and rice seeds. Due to the rapid depletion of oxygen in seed tissues upon imbibition, oxidative respiration becomes limited, resulting in the depleted energy status of early germinating seeds. The ATP molecules produced by cellular respiration provide the energy for seed germination to begin and fuel the cell-building activities that ultimately form the plant body, and we have noticed differential expression in date palm seeds.

We also observed differential gene expression in response to hydrogen peroxide in some microgravity date palm seeds. Hydrogen peroxide (H_2O_2) promotes seed germination in cereal plants, and ascorbic acid, which acts as an antioxidant, suppresses germination. Reactive oxygen species promote the release of seed dormancy by biomolecule oxidation, testa weakening, and endosperm decay. Reactive oxygen species modulate metabolic and hormone signaling pathways that induce and maintain seed dormancy and germination. Reactive oxygen species (ROS) have traditionally been viewed as destructive agents in plants; however, it has been recently shown that ROS also play a positive role in seed germination. Oxygen can be transformed into multiple ROS including singlet oxygen, superoxide, hydroxyl radicals, and hydrogen peroxide. Hydrogen peroxide is considered an important ROS agent because it can readily pass through various cellular membranes. Reactive oxygen species production inside the seed transforms it from a quiescent seed produced by the mother plant into a biologically active seed that is capable of germination (Xia et al., 2018). This process occurs when environmental signals are accurately perceived and processed into endogenous signals by the seeds (Xia et al., 2018).

Thus, radiation and vibration damage both living and non-living components in several ways. We found that these factors affected the seeds. While faster germination may seem inconsequential or even beneficial when the result for a seed is germination, we advise that the effects of radiation and vibration on crop seeds warrant further consideration when planning long-term storage and space travel for three reasons. First, continued disruption of the protective seed coat during protein production likely leads to micro-fissures on the seed surface. During dormancy, these fissures can hasten water loss and/or degrade the embryo food source, leading to seed death. Second, germination differs in viability. Physiological or genetic damage to embryos during dormancy or seed formation may not manifest until the plant grows. This study focused on germination, not viability, to evaluate the effects of space travel on the long-term storage/use of seeds. Third, rapid germination does not always produce robust seedlings. Associated changes in seedling growth and enzymatic function should be studied, particularly if seed crops are intended for food production far from Earth's home in outer space.

Chapter 6: Conclusion

Growing in space is a target project of the UAE, although it is difficult, costly, and requires many experiments and time. Because of plans to use plants as an integral component of life support systems for human outposts in space, seed biology has become a topic of critical importance. Microenvironments within plant structures, such as the air space around seeds inside a pod, also change in the spaceflight environment. These changes have adverse effects on seed quality and thus have important consequences for the role of plants in bioregenerative life support systems as well as implications for dynamics within the microenvironment of the developing seed. The resilience of dry seeds was revealed in exposure experiments to a harsh space environment in which their viability was maintained for a considerable time if shielded against short-wave UV irradiation. We hypothesize that the observed seed aging during space travel, which included six months of dry storage onboard the ISS, is caused mainly by exposure to low-level ionizing radiation from galactic cosmic rays, trapped protons, and solar energetic particles, perhaps in combination with mechanical vibration. The absorbed radiation dose on board the ISS is 100-times more than that on the Earth's surface and affects the seed transcriptome, germination physiology, and aging sensitivity. However, long-distance spaceflights would cause much higher exposures for seeds as well as for humans. However, seed storability and aging resilience differ considerably between seed lots, cultivars, and species, depending on the crop genotype and seed production environment. Thus, careful consideration of seed material, reducing mechanical vibration, and shielding from space radiation would be prudent when taking dry seeds beyond Earth's orbit, although this study and many others suggest that the goal of growing crops in other worlds is achievable.

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Appendix

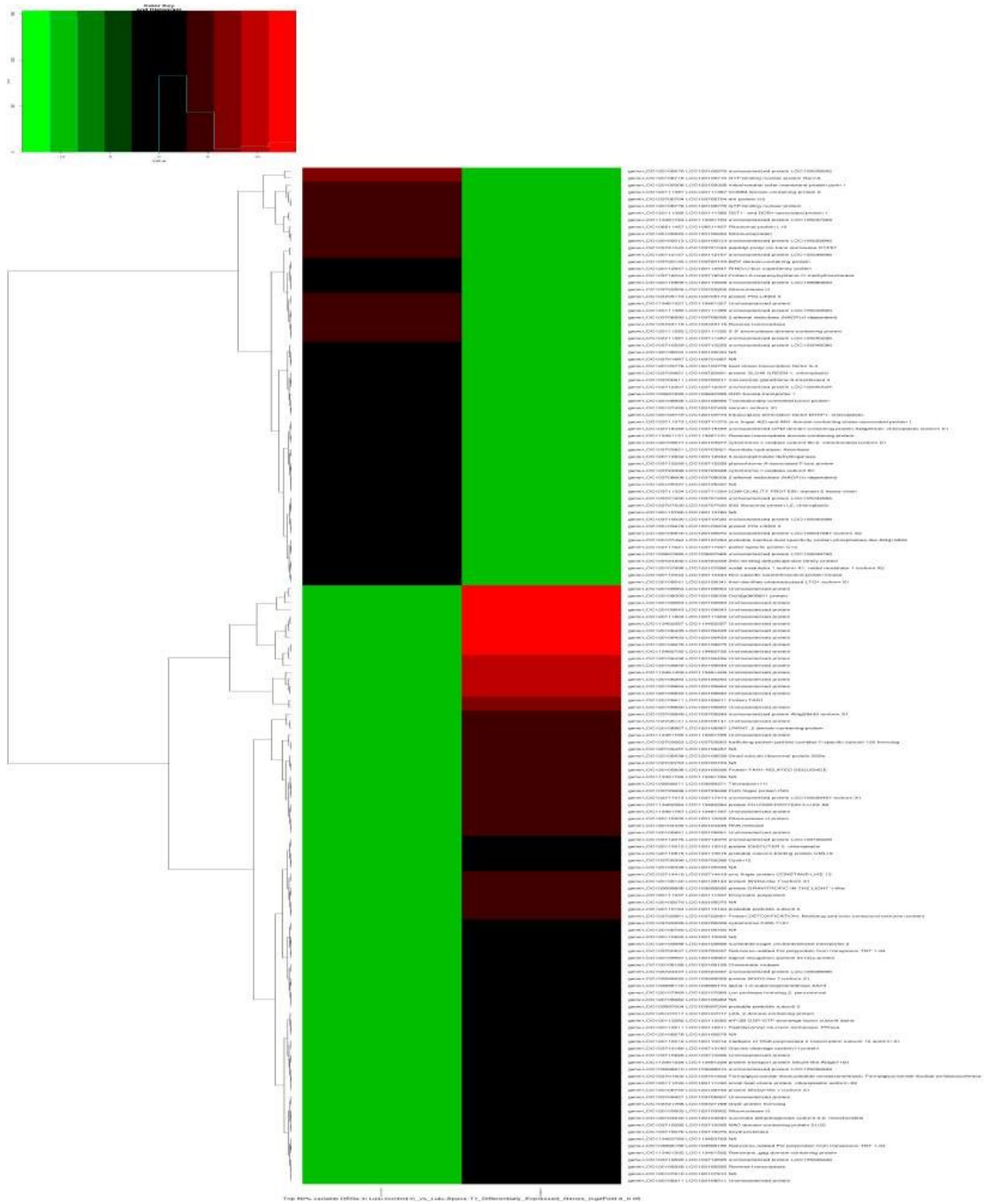


Figure 1: Heat maps showing differential gene expression in the KEGG pathway for Lulu control vs. Lulu space of the top 50% differentially expressed genes.

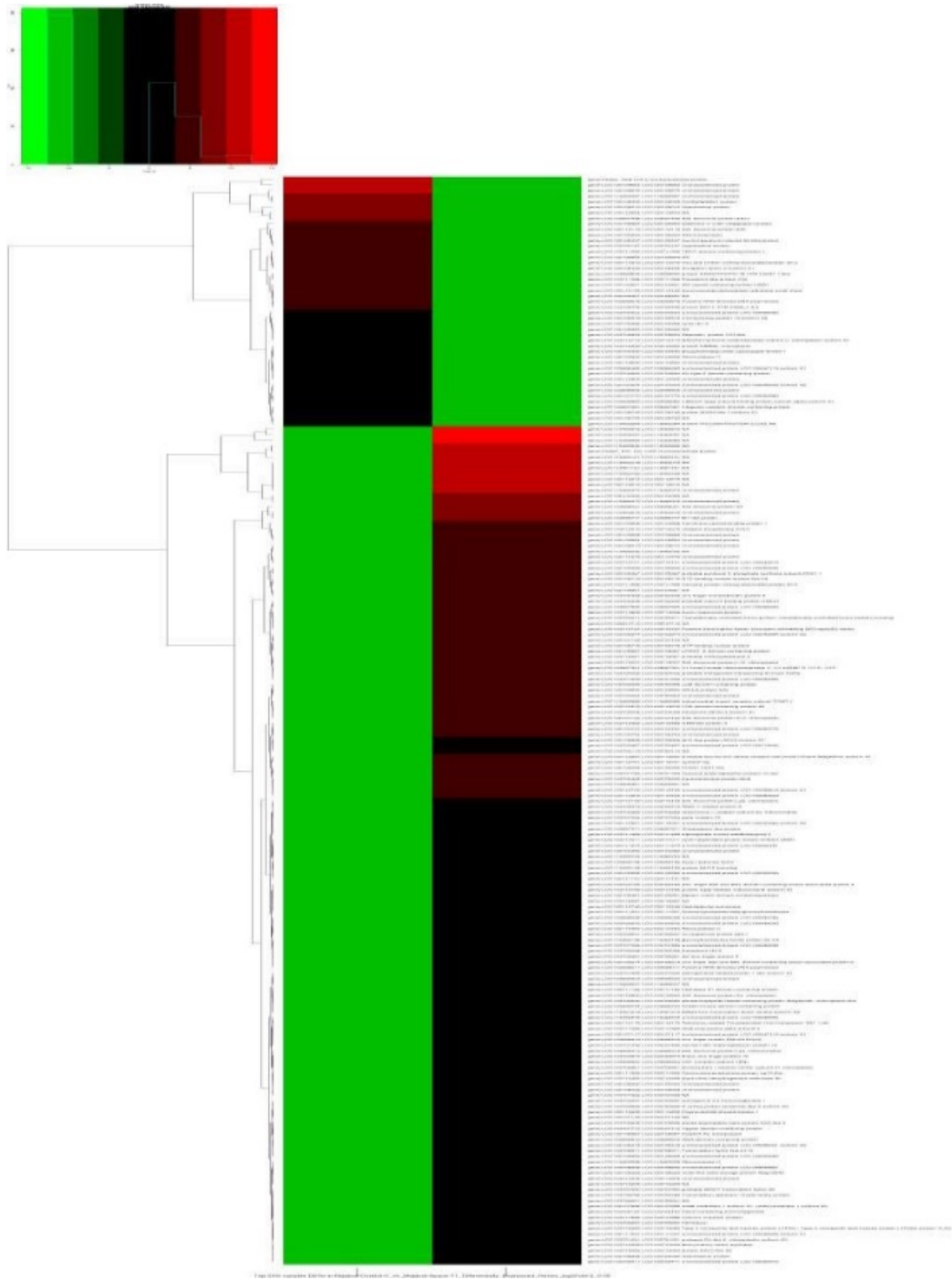


Figure 2: Heat maps showing differential gene expression in the KEGG pathway for Majdool control vs. Majdool space of the top 50% differentially expressed genes.

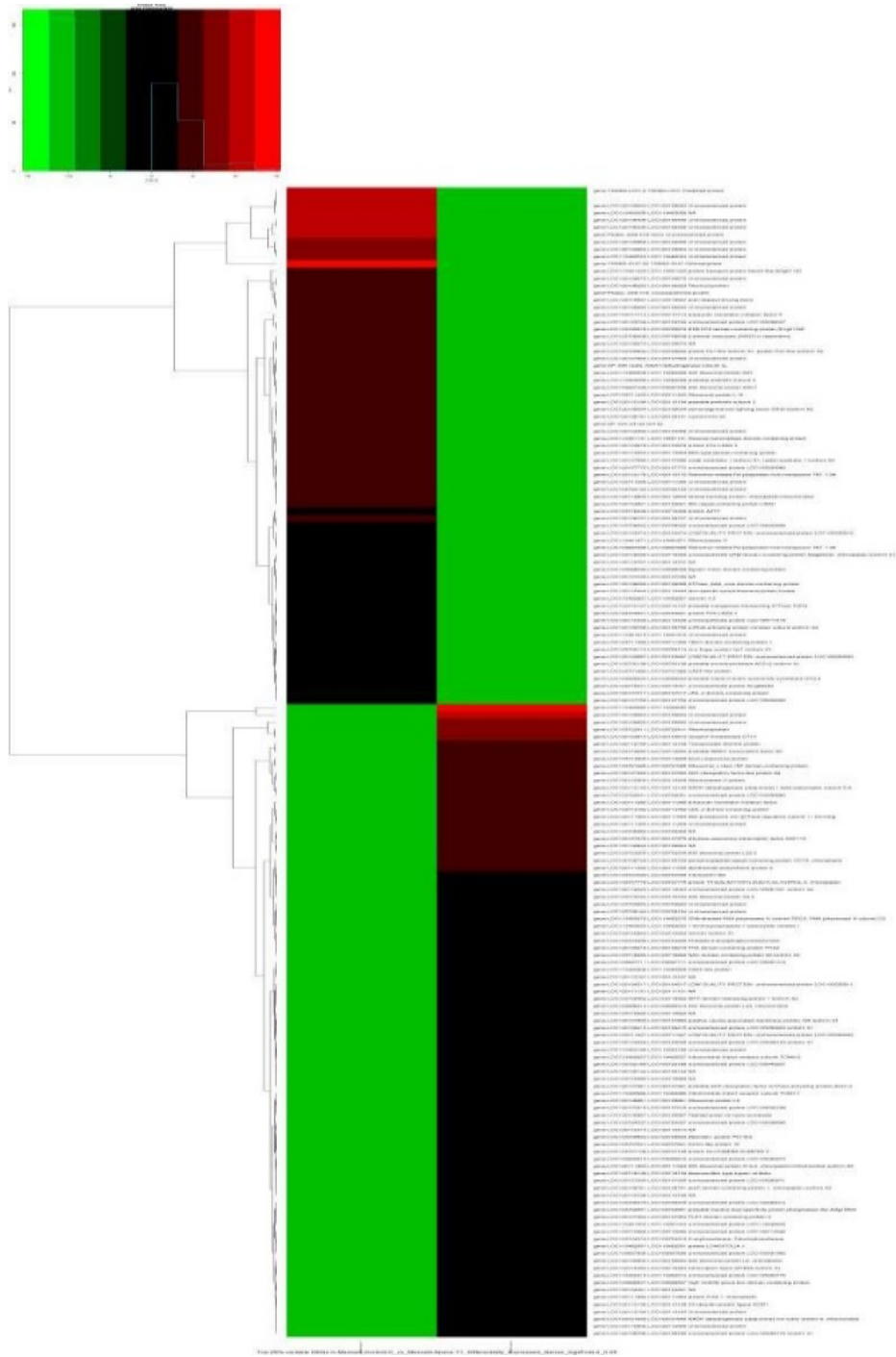


Figure 3: Heat maps showing differential gene expression in KEGG pathway for Mesalli control vs Mesalli space of the top 25% differentially expressed genes.

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The proposed research focuses on basic research to complement the UAE initiative to send date palms to Mars for future space missions. Plant growth analysis of date palm seeds was performed after maintaining at zero gravity in outer space in the International Space Station (ISS) and simultaneously under normal gravity at ground level. In this context, this work was carried out to analyze the molecular changes in the date palm seeds maintained at microgravity in comparison with the gravity seeds. Ninety seeds of different varieties of date palm were sent to space in collaboration with the UAE space agency for 6 months. Many experiments were done to know what exactly happened while at zero gravity.

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