



Faculty of Resource Science and Technology

**Comparative Leaf Proteomes of Irradiated Mutant and Wild Type *Oryza sativa subsp. indica***

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Bachelor of Science with Honours  
(Resource Biotechnology)  
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**Comparative Leaf Proteomes of Irradiated Mutant and Wild Type *Oryza sativa subsp. indica***

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A thesis submitted in partial fulfilment of the Requirement of The Degree Bachelor of  
Science with Honours  
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Programme of Resource Biotechnology  
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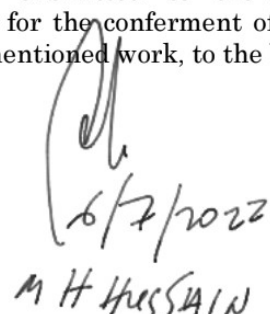
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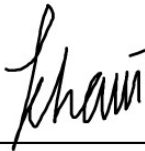
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# Comparative Leaf Proteomes of Irradiated Mutant and Wild Type *Oryza sativa subsp. indica*

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## ABSTRACT

Radiation is a common phenomenon that occurs in the natural setting which leads to mutation occurrence in various organisms. In the plant kingdom, particularly, the occurrence of mutation is unnecessarily reflected as a negative thing. However, for over centuries, humanity has inverted perspectives on mutation by labelling mutation as a defect in any organism and conjecturing mutation solely gave disadvantages for humankind. Therefore, this study adapted the proteomics approach in investigating the comparison between irradiated mutant and wild type of *Oryza sativa subsp. indica* at proteome level. The leaf samples were prepared by using a conventional method of grinding in liquid nitrogen by using mortar. The adoption of PEG fractionation technique in this study ensured an efficacious way of protein retrieval from samples. The use of Bradford protein assay provided a close approximation of protein concentration in the sample. The adoption of SDS-PAGE helped in separation of proteins according to their molecular weight. The gel result from SDS-PAGE revealed that separation of 13 proteins from the supernatant sample ranged from 10 to 74 kDa for both rice types whereas for the pellet sample separated 8 proteins ranged from 10 to 45 kDa. In addition, pellet samples for both rice types are unable to resolve a clear band separation due to low protein abundance with 5 protein bands unable to be detected as compared to supernatant samples. The obvious difference only the band thickness at Band 5 (42 kDa) between wild type and mutant type which suggested there is alteration of protein production occurred in mutant type.

**Key words:** Proteomics, rice plant, *Oryza sativa subsp. indica*, mutation, SDS-PAGE.

## ABSTRAK

Radiasi merupakan fenomena biasa yang sering berlaku dalam persekitaran semula jadi yang menyebabkan terjadinya mutasi dalam pelbagai organisma. Terutamanya dalam tumbuhan, kejadian mutasi tidak semestinya menggambarkan perkara yang negatif. Walaubagaimanapun, manusia mempunyai perspektif yang terbalik mengenai mutasi dengan melabel mutasi dengan ketidaksempurnaan dalam mana-mana organisma dan beranggapan mutasi memberi keburukan kepada manusia semata-mata selama berabad-abadnya. Oleh itu, kajian ini mengambil pendekatan proteomik dalam menyiasat perbandingan antara jenis irradiasi mutan dan jenis biasa daripada *Oryza sativa subsp. indica* di tahap proteom. Penyediaan sampel-sampel daun menggunakan kaedah konvensional dengan mengisarnya dalam nitrogen cecair dengan menggunakan mortar. Penggunaan teknik pecahan PEG dalam kajian ini memastikan cara yang berkesan untuk memperoleh protein dari sampel-sampel. Penggunaan ujian protein Bradford menyediakan anggaran kepekatan protein yang dekat dalam sampel. Penggunaan SDS-PAGE membantu dalam pemisahan protein mengikut berat molekul. Hasil gel dari SDS-PAGE memperlihatkan pemisahan 13 protein dari sampel supernatan yang berjulat antara 10 hingga 75 kDa untuk kedua-dua jenis beras manakala untuk sampel pellet pula memisahkan 8 protein yang berjulat antara 10 hingga 45 kDa. Di samping itu, sampel pellet untuk kedua-dua jenis beras tidak dapat untuk menghasilkan pemisahan jalur yang jelas kerana protein kepekatan protein yang rendah dengan 5 jalur protein tidak dapat dilihat berbanding dengan sampel supernatan. Perbezaan yang ketara hanyalah ketebalan jalur di Jalur 5 (42 kDa) antara jenis biasa dan jenis mutan yang menandakan terdapat perubahan dalam penghasilan protein berlaku dalam jenis mutan.

**Kata kunci:** Proteomik, tanaman padi, *Oryza sativa subsp. indica*, mutasi, SDS-PAGE.

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## LIST OF ABBREVIATIONS

%	Percentage
°C	Degree of Celsius
g	Gram
M	Molar
V	Volt
mM	Millimolar
mL	Millilitre
µL	Microlitre
µg	Microgram
×g	Times gravity
pH	Potential of hydrogen
v/v	Volume per volume
m/v	Mass per volume
Mg	Magnesium
HCl	Hydrochloric acid
MgCl <sub>2</sub>	Magnesium chloride
SDS	Sodium dodecyl sulphate
TCA	Trichloroacetic acid
PEG	Polyethylene glycol
CBB	Coomassie Brilliant Blue
DTT	Dithiothreitol
Rubisco	Ribulose-1,5-bisphosphate carboxylase-oxygenase
PMSF	Phenyl methyl sulfonyl

SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
2D-PAGE	Two dimensional-polyacrylamide gel electrophoresis

## CHAPTER 1

### INTRODUCTION

*Oryza sativa* or universally known as rice, is a type of grass that bears grains and known for its close relation to *Oryza rufipogon*, which is a wild grass in Asia. Depending on their subspecies, *O. sativa* is quite versatile crops that can be easily grown in various types of area, including highland, lowland, and irrigated or flooded areas (UniProt, 2021). Over centuries of years, human civilization manifested their dependency on rice as one of the most important crops that contributed to the primary source of food for most of the global population (Callaway, 2014).

The rice subspecies, *indica*, is typically grown in the tropical and subtropical regions (UniProt, 2021). It is a very popular rice subspecies that is suitable to be grown in well-irrigated areas such as in Southeast Asia and Southern China region (Ricepedia, n. d.). Especially in Southeast Asia, *indica* rice is recognized as socio-economically valuable and has become the staple food for most Asian countries. In realising primacy of *indica* rice, numerous research studies actively conducted to produce desirable traits of rice.

Although scientific studies on *indica* rice were well-conducted, there is still a lack of scientific studies focusing on the perspective of rice proteomics, which mostly related to plant tolerance to certain stresses such as high temperature, salt, drought, and the trace element. It also majorly focused on the comparative proteomes study between rice plant subspecies, *indica* and *japonica* rice. Apparently, there is a lack of comparative proteomics study that basically investigates the comparative analysis between the mutant and wild type of *indica* rice.

Over the past several decades, proteomics has become an influential field that is extensively utilised in scientific research. Proteomics offers a very powerful tool in scientific research which provides a high-throughput identification of protein (Thelen, 2007) following with high accuracy of analysis. In addition, the assistance by various types of compatible advancement, such as separation of protein, genome sequencing and spectrometry (Thelen, 2007) pointed to proteomics as a versatile field. In this study, proteomics is adapted in assisting the investigation of comparative study between two types of *indica* rice. The work was initiated by applying PEG fractionation protocol to extract the protein from pulverised leaves samples. The extracted samples were then subjected to a quantification procedure by applying Bradford assay. Finally, the quantified samples are then analysed by running SDS-PAGE analysis for separation of protein.

All the plants in the wild environment are exposed to various types of radiation. A concern may arise which highlights the issue of rice plant produce due to irradiation exposure. Therefore, this study will facilitate in determining whether the mutant type of *indica* rice gives total defect or absolute advantage for the agricultural industry. Hypothetically, when a rice plant is exposed to radiation in wild environment, there will be a difference in protein expression. Therefore, the comparison of leaf proteomes between irradiated mutant and wild type *indica* rice would reveal the differences between irradiated mutant type as compared to wild type.

Objectives:

- To compare the leaf proteomes between irradiated mutant and wild types of *O. sativa subsp. indica*.
- To evaluate the difference of protein band between irradiated mutant type and wild type of *O. sativa subsp. indica*.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1. The history of rice cultivation

The cultivation of rice has an intricate history and remains debatable from time to time. Human civilizations witnessed the significant role of rice as the most prominent source of food over centuries. In fact, Asian civilization was developed based on the rice, *Oryza sativa* (Callaway, 2014). From the historical records, rice remarkably led to a major type of transformation on humans which was responsible in shifting nomadic hunters and gatherers into the permanent farmers (Callaway, 2014) that depending on agricultural activities. This transition is believed to be one of the core reasons that spawned a rapid development of empires and dynasties. It is supported by an archaeobotanist at University College London, Dorian Fuller, who emphasised rice as the most likely crop which led to various types of transformations for societies, economies, and landscape (Callaway, 2014).

Above all, the domestication of rice was the most important historical event. It is believed that the domestication of rice is centred in the Asian region and mostly in tropical and subtropical regions. An evolutionary geneticist from New York University, Michael Purugganan stated that in the Asian region, almost every part of the region is linked with the origin of rice (Callaway, 2014). The rice domestication is believed been taken approximately 9000 years (Oka, 1988, as cited in Wang et al., 2017) since its derivation from wild grass species, *Oryza rufipogon* (Fuller et al., 2007, as cited in Wang et al., 2017). Due to the primacy of rice, there are numerous debatable arguments that vigorously emerged and



claimed the rice origin came from their nation. Despite these contrary viewpoints, archaeological evidence has been enlightening the fact behind the controversy of rice domestication.

As the data obtained from the archaeological analysis, they suggested that China and India are the two nations particularly that are strongly pointed as the centre of rice species domestication. Particularly, the archaeological evidence firmly points that China is the most probable centre of domestication of japonica rice that have been cultivated around 9000 years BP and specifically in the lower Yangtze Valley (Gutaker, 2020). Based on historical events, they were also emphasised that the spread of japonica rice into the Indian region was the initial point of indica rice emergence where probably due to the unintentional hybridization (Callaway, 2014). In detail, back to 4000 years BP, the widespread of japonica rice from East Asia to South Asia became the main reason that drove the incorporation of japonica alleles into the local *Oryza nivara* population by unintentional means. This event then led to the emergence of a new type of rice which is indica (Huang et al., 2012; Choi et al., 2017; Choi et al., 2018 as cited in Gutaker et al., 2020).

## **2.2. Botanical features of rice**

Rice plants are grouped in Poales order and classified under the Poaceae family (Mia, 2016). Rice is a type of annual plant which completes its life cycle starting from germination to seed production within one growing season. Specifically, rice plants can also be classified under monocarpic plants which have only one flowering season to set their seeds before eventually die (Tripathi et al., 2011). Simply, it is a type of plant that only grows and produces seeds once along their life. In general, rice plants consist of three basic systems which are the root system, the shoot system, and the flower system.

There are three forms of roots that are further classified into two groups of major root type which are nodal roots and crown roots (Tripathi et al., 2011). These two major root types can be distinguished according to their node's position where they arise from. The roots which arise from nodes situated above the ground are called nodal roots. On the contrary, crown roots are the roots that developed from nodes that were situated below the ground. The crown roots then can be further classified into two types of roots which are mat roots and ordinary roots. The mat roots are covering the soil area near to the surface whereas the ordinary roots cover the deeper soil area.

Basically, the stem accounted for the main structure of the rice plant. It consists of two parts, the aerial and underground stem (Tripathi, 2011). Generally, the rice stem is characterised as a hollow and rounded or a cylindrical shape which resembles more like a straw (Albert, 2017). There are presence of nodes and internodes along the stem and often called as culm (Mia, 2016). The node is where the buds and leaves arise from. Initially, the first node will develop the first leaves that cover the main culm (Tripathi, 2011). The main culm or central stem (Albert, 2017), also called as a primary tiller is a projection from the main stem. At a later stage, there will be a development of secondary tillers from the first nodes to primary tiller (Tripathi, 2011).

Morphologically, the rice leaf can be characterised as a simple, flat sheath, long size and blade-like structure. The leaf's sheath is attached on the main stem that encircled it with an alternative arrangement (Mia, 2016; Tripathi, 2011). Simply, the leaves sprouted entirely much like grasses. The buds enclosed between the leaf and stem are protected by this leaf sheath (Albert, 2017). The leaf's sheath is attached to the main stem that encircled it with an alternative arrangement (Mia, 2016; Tripathi, 2011). In rice, the flower is known as floret. It is a bisexual and zygomorphic reproductive organ which consisted of bract lemmas and palea (Mia, 2016). In detail, the floral structure of rice comprises two lodicules which are

characterised as small, thick, oval, and fleshy bodies of structure. The lodicules will swell and create an opening as the way of exposing their matured floral parts (Tripathi, 2011). At the centre of the floret, there are six stamens with twirly arrangement and a pistil presence. The bilobed anther that acts as the storage part of pollen grains (Tripathi, 2011) is held by a twirly filament. The pistil is surrounded by these six stamens and consists of ovary, style and stigma (Tripathi, 2011).

### **2.3. Basic anatomy of rice grain**

The seed of the rice plant, or often called as the grains is developed when the pollination and fertilisation events were succeeded. In the usual scenario, the grain took about 35 days to ripen and mature after the pollination event (Albert, 2017). This dry one seeded fruit possesses similar basic anatomy regardless of what species they originated from. There are four major parts of a rice grain which are hull, bran, endosperm, and germ as shown in Figure 1 below. All grains from different rice species also share similar characteristics such as the presence of tough and hard outer layers as their protection from outside threats such as insect invasion. This protection layer or called as the hull is fused with a pericarp layer and comprises several parts which are a lemma, a pair of sterile lemmas, a palea, a grain stem which is called as the rachilla and a tail which is called as awn. Particularly, the hull is a fibrous hard cover that accounts for 20% of the total weight of a grain. To turn them into edible rice, the hull is removed (Tripathi, 2011).

The bran layer, or sometimes called an aleurone layer comes from after the seed coat or testa. The endosperm accounts for the biggest area of a rice grain and the embryo located in the inner part of the endosperm. Apparently, it is the first layer of the edible part of a grain. This brownish layer accounts for 8% of the total weight of a grain and is rich in oil, vitamins,

and minerals. It also acts to enclose the inner part of the seed which endosperm and embryo. The biggest area of rice grain is dominated by the endosperm which takes 70% of the total weight of a grain. The endosperm contains high starch, protein, and fat. In a small area of the inner part, there is rice embryo present that accounts for 2% of the total weight of a grain and is able to continue sprouting into a new plant thus needing to be removed in the rice processing (Tripathi, 2011).

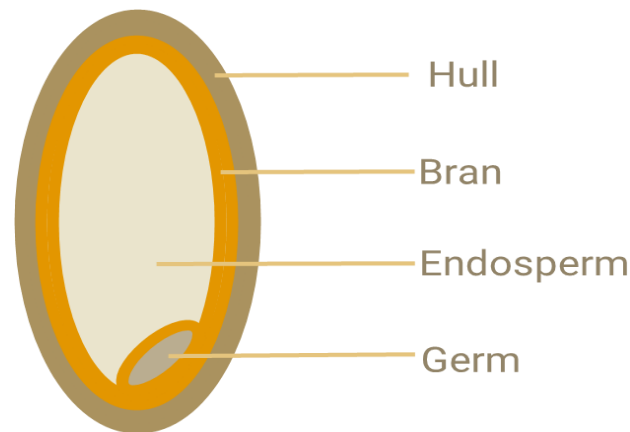


Figure 1: Four major parts of a rice grains

Picture prepared by Mohd Khairul Akma Darwis

#### **2.4. *Oryza sativa* L. subspecies *indica***

The variation of *Oryza* genus comprises six genomes of diploid chromosomes and five genomes of allotetraploid chromosomes (Vaughan et al., 2003, as cited in Chin et al., 2016). These eleven genomes then subdivided and formed 24 species of *Oryza* genus (Marathi, et al., 2015 as cited in Chin et al., 2016). According to Chin et al. (2016), in the rice evolution, the emergence of two subspecies of *O. sativa* which are subspecies *indica* and *japonica* is considered a breakthrough in agriculture history. This review will focus more on the perspective of *indica* subspecies.

Morphologically, *indica* rice plant is not much different from other rice species. Morphologically, *indica* rice is a tall grassy plant. The leaves are light green in colour which have heavy tillering bladed leaves. The leaf itself can be characterised as a long-bladed leaf which ranges from broad to narrow. There is quite a variation of *indica* rice grains. The *indica* rice plant is well-known to bear grains that long and thin in size. But there are also *indica* rice plants that bear grains that are short and thick in size. *Indica* rice grains also can be shattered easily and have high content of amylose. The grains are a bit drier and when cooked, rice is much flakier as compared to the japonica grains (Ricepedia, n. d.).

Typically, *indica* rice plants are grown in the tropical and subtropical regions of the globe such as Southeast Asia and Southern China (UniProt, 2021). As the plant is well-adapted to the excessive amount of water above the normal range, it is then suitable to be grown in well-irrigated areas. In fact, *indica* rice may not survive when grown in typical soil. *Indica* rice is now considered as the most prominent type of rice among all 24 rice species. This can be proven from the statistics of world rice cultivation where the cultivation of *indica* rice accounted for over 80% of rice cultivation in the world (Mahesh et al., 2016).

Bario rice is a variety of *indica* rice and well-known as a cultivar that come from Sarawak, Malaysia. Usually, barrio rice variety is well-adapted to highland environment. It is considered as one of the finest grains in the world because of its uniqueness for having soft texture, fine and elongated grains, exquisite taste and full of aromas (Wong et al., 2009). Although of that impressive fact, somehow it is opposite with the lack of research efforts that have been conducted for barrio rice variety. Therefore, realising this phenomenon, this study is intended to take barrio rice variety as the main subject for the investigation.

## 2.5. Understanding comparative proteomics study

A rapid advancement of biotechnology led to the emergence of various types of essential subdivision, including the proteomics that become a guideline for researchers nowadays. Conspicuously, protein is the base of the proteomics study. Protein was first described by Jons Jakob Berzelius, a Swedish chemist in 1838 in his intention of describing the molecules that are prominent in supporting living organisms, subsequently named it as protein (Aslam et al., 2016). Protein is adapted from the Greek word which is *proteios* that means “the first rank” (Cristea et al., 2004 as cited in Aslam et al., 2016). Marc Wilkins initially denoted the term proteomics to refer to the “protein complement of a genome” in 1996 (Aslam, 2016). Since then, the term “Proteomes” was widely spread in the scientific field.

Generally, proteomics is the core study of protein which includes protein content in organisms, protein interaction, protein expression and protein modification. The term of proteomics then is narrowed down by the subdivision of comparative proteomics study. Basically, comparative proteomics is utilised in most of the protein studies that provide a clear vision for researchers on the protein differentiation in terms of their content, expression, interaction, and modification. In a way, comparative proteomics covers a wide range of proteomics subdivisions whereby the major objective is to inspect any changes associated with protein in an organism.

For the past several decades, comparative proteomics has facilitated researchers in understanding various aspects of scientific mechanisms in living organisms such as physiological processes, biochemical pathways, and adaptation mechanisms. In addition, comparative proteomics also aids in the pedigree analysis of species as well as differentiation

between species. In the plant kingdom, particularly, there are abundant useful studies that have been conducted by adopting comparative proteomics as the main approach.

For instance, in a journal published by BMC Genomic, a study conducted by Zhan et al. (2019) that focused on okra (*Abelmoschus esculentus*) seedlings use proteomics analysis in understanding the salt tolerant response in okra. From the study, they have identified 7179 proteins in total that are associated with several annotated categories such as biological processes, molecular functions, and cellular components. The comparative proteomics aids in the detection of 317 in total with differentially expressed proteins (DEPs). Further comparative proteomics analysis then revealed, out of 317 of DEPs, there are 165 upregulated proteins and 152 downregulated proteins that have been detected when treated with higher NaCl concentration.

On a similar note, Sithtisarn et al. (2017) provided a clear insight of reference involving comparative proteomics in the Protista kingdom by using green algae to understand the salinity tolerance. For this study, they used green algae, *Chlamydomonas reinhardtii* as the model study. Before using proteomic analysis, they developed a model that has a salinity-tolerance strain that is able to grow in a high salinity environment. They adopted comparative proteomics approaches such as SDS-PAGE, in-gel tryptic digestion and LC-MS/MS. From the findings, they detected 93 upregulated proteins and 34 down regulated proteins. They suggested that upregulated proteins have an imperative role in transforming green algae into salinity-tolerant organisms.

A quite similar study in plant response on abiotic stress has been conducted by Zenda and coworkers (2018) that took maize (*Zea mays*) seedlings as the model organism. The study in purpose of revealing an extensive comparative analysis in physiology and proteomes between two maize inbred lines which are YE8112 and MO17 when dealing with

drought stress. In this study, a comparative proteomics analysis between two inbred lines was conducted after a week of moisture-deficit exposure. They found that there are 721 of differentially abundant proteins (DAPs) in total. For the further analysis of the combination between proteomes and physiology, they revealed that the drought tolerance of YE8112 is associated with the activation of photosynthetic proteins. These photosynthetic proteins are involved in several physiological and biochemical mechanisms that become the core reason of drought tolerance in YE8112 maize breed line.

Comparative proteomics has also been useful for understanding the molecular basis of horseweed resistance on glyphosate. The study conducted by Gonzalez-Torralva and coworkers (2017) indicated that an overlapping of proteins when treated with glyphosate and untreated with glyphosate. Primarily, there is no solid evidence that these proteins are involved in herbicide resistance of horseweed, but based on the use of comparative proteomics analysis, they are able to conjecture due to the subcellular localization of some of these proteins attributed to glyphosate resistance.

## **2.6. Comparative proteomics study on rice**

The comparative proteomics study also conducted in rice plants in understanding the physiological, biochemical, and adaptation of rice. Specifically, it is focusing more on the comparative analysis of protein that is associated with rice adaptation. For instance, comparative proteomics study has been adopted by researchers in investigating the tolerance in plants such as drought, salinity, high temperature and trace elements. For example, proteomics analysis of the rice plant under drought stress that conducted by Chintakovid and coworkers (2017) have sorted out several types of proteins that involved in retrotransposon, transcription and post transcription as well as transportation, by which prominently