TEMPERATURE, VACUUM PACKAGING AND PRIMING EFFECTS ON GERMINATION AND STORABILITY OF NEW BREED SWEET CORN (*Zea mays* var. GSH11005Y) SEED

By

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The increasing demand for sweet corn has highlighted the need for improved seed production, but challenges such as low germination rates and poor storability have hindered large-scale cultivation. To address these, three studies were conducted to explore factors affecting seed quality and longevity. In the first study, GSH11005Y sweet corn seeds were harvested at five maturity stages (29, 32, 35, 38, and 41 days after pollination, DAP) and dried at temperatures of 35°C, 40°C, and 45°C. Seeds harvested at 29–35 DAP showed the best seed vigour, with drying at 40°C yielding the highest seedling performance and maintaining seed viability (>90%). Drying at 45°C led to seed damage, indicated by elevated malondialdehyde (MDA) levels and reduced seedling vigour. Antioxidant enzyme activities, including catalase (CAT) and peroxidase (POD), varied by maturity stage and drying temperature, with 40°C drying enhancing POD activity. The second study examined the effects

of packaging type (full vacuum, partial vacuum, airtight sealed) and storage temperature (16°C and 26±3°C) on seed longevity. Vacuum packaging at 16°C effectively maintained seed viability, with germination percentages remaining above 80% after one year, while seeds stored at 26±3°C in vacuum packaging saw a significant decline in viability (22–26%). POD activity was higher in seeds stored at 16°C, indicating better seed health under these conditions. The third study investigated hydrogen peroxide (H_2O_2) priming on aged seeds, using concentrations ranging from 1 mM to 20 mM for 24 hours. Priming with 10 mM H_2O_2 resulted in a significant increase in seed viability (72%) and germination speed, while higher concentrations (above 15 mM) showed no additional benefits. Reduced MDA levels and lower electrical conductivity of seed leachate further confirmed the improvement in seed quality. Collectively, the studies suggest that sweet corn seeds harvested at 29 DAP are suitable for immediate use, while 35 DAP is recommended for storage. Drying at 40°C optimizes seed quality and storability by reducing moisture content and enhancing antioxidant enzyme activity. Vacuum packaging at 16°C provides the best conditions for long-term storage, while priming with 10 mM H_2O_2 effectively rejuvenates aged seeds, improving germination, vigour, and seedling establishment.

Keywords: Seed Technology, Seed Priming, Vacuum Packaging, Sweet Corn Seed, Seed Drying.

SDG: GOAL 01: No Poverty, GOAL 02: Zero Hunger

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN SUHU, PEMBUNGKUSAN VAKUM DAN PRIMING KE ATAS PERCAMBAHAN DAN PENYIMPANAN BIJI BENIH JAGUNG MANIS BAKA BARU (*Zea mays* var. GSH11005Y)

Oleh

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Permintaan yang semakin meningkat untuk jagung manis telah mendesak keperluan untuk pengeluaran biji benih yang lebih baik, tetapi wujudnya cabaran seperti kadar percambahan yang rendah dan kadar penyimpanan yang lemah telah menghalang penanaman berskala besar. Untuk menanganinya, tiga kajian telah dijalankan untuk meneroka faktor yang mempengaruhi kualiti dan jangka hayat biji benih. Dalam kajian pertama, biji benih jagung manis GSH11005Y dituai pada lima peringkat kematangan (29, 32, 35, 38, dan 41 hari selepas pendebungaan, DAP) dan dikeringkan pada suhu 35°C, 40°C, dan 45°C. Benih yang dituai pada 29–35 DAP menunjukkan kekuatan benih terbaik, dengan pengeringan pada suhu 40°C menghasilkan prestasi anak benih tertinggi dan mengekalkan daya maju benih (>90%). Pengeringan pada suhu 45°C membawa kepada kerosakan benih, ditunjukkan oleh tahap malondialdehid (MDA) yang tinggi dan tenaga anak

benih yang berkurangan. Aktiviti enzim antioksidan, termasuk katalase (CAT) dan peroksidase (POD), diubah mengikut peringkat kematangan dan suhu pengeringan, dengan pengeringan 40°C meningkatkan aktiviti POD. Kajian kedua mengkaji kesan jenis pembungkusan (vakum penuh, vakum separa, kedap udara) dan suhu penyimpanan (16°C dan 26±3°C) pada umur panjang benih. Pembungkusan vakum pada suhu 16°C dengan berkesan mengekalkan daya maju benih, dengan peratusan percambahan kekal melebihi 80% selepas satu tahun, manakala benih yang disimpan pada suhu 26±3°C dalam pembungkusan vakum menyaksikan penurunan kesegahan biji benih yang ketara (22-26%). Aktiviti POD adalah lebih tinggi dalam benih yang disimpan pada suhu 16°C, menunjukkan kesihatan benih yang lebih baik di bawah keadaan ini. Kajian ketiga menyiasat rawatan priming menggunakan hidrogen peroksida (H_2O_2) pada benih lama, menggunakan kepekatan antara 1 mM hingga 20 mM selama 24 jam. Priming dengan 10 mM H_2O_2 menghasilkan peningkatan ketara dalam kesegahan biji benih (72%) dan kelajuan percambahan, manakala kepekatan yang lebih tinggi (melebihi 15 mM) tidak menunjukkan faedah tambahan. Tahap MDA yang dikurangkan dan kekonduksian elektrik yang lebih rendah mengesahkan peningkatan dalam kualiti biji benih yang lama. Secara kolektif, kajian menunjukkan bahawa benih jagung manis yang dituai pada 29 DAP adalah sesuai untuk kegunaan segera, manakala 35 DAP disyorkan untuk disimpan. Pengeringan pada suhu 40°C mengoptimumkan kualiti dan kadar penyimpanan biji benih dengan mengurangkan kelembapan biji benih dan meningkatkan aktiviti enzim antioksidan. Pembungkusan vakum pada suhu 16°C menyediakan

keadaan terbaik untuk penyimpanan jangka panjang, manakala priming dengan 10 mM H_2O_2 berkesan memulihkan benih tua dengan meningkatkan percambahan, cergas dan pembentukan anak benih.

Kata Kunci: Teknologi Biji Benih, Priming Biji Benih, Pembungkusan Vakum, Biji Benih Jagung Manis, Pengeringan Biji Benih.

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LIST OF ABBREVIATIONS

°C	Degree Celsius
ABA	Abscisic Acid
ANOVA	Analysis of Variance
ATS	Air-tight Sealed
BOT	Balance of Trade
CAT	Catalase
cm	centimetre
CVG	Coefficient Velocity of Germination
DAA	Days After Anthesis
DAP	Days After Pollination
DAS	Days After Silking
DOA	Department of Agriculture
DW	Dry weight
FAO	Food and Agriculture Organization
FW	Fresh Weight
FGP	Final Germination Percentage
FV	Full Vacuum
FW	Fresh weight
g	gram
GA	Gibberellic Acid
GI	Germination Index
GRI	Germination Rate Index
GWG	Green World Genetic
H_2O_2	Hydrogen Peroxide
IAA	Indole-3-acetic Acid
ISTA	International Seed Testing Association
kg	Kilogram
MARDI	Malaysia Agriculture Research and Development Institute
MAS	Month After Storage
MC	Moisture Content
MDA	Malondialdehyde

mg	milligram
MGT	Mean Germination Time
PE	Polyethylene
PM	Physiological Maturity
POD	Guaiacol Peroxidase
PPC	Per Capita Consumption
PV	Partial Vacuum
RH	Relative Humidity
ROS	Reactive Oxygen Species
Se1	*Sugary1*
RQ	Respiratory Quotient
RH	Relative Humidity
Sh2	*Shrunken2*
SSR	Self Sufficiency Ratio
SVI	Seedling Vigour Index
SOD	Superoxide Dismutase
TEMP	Temperature

CHAPTER 1

INTRODUCTION

Corn (*Zea mays* Linn.), belonging to the family *Poaceae,* is among the largest field crop planted worldwide as it can be grown in diverse climates. Various types of corn are cultivated such as dent, flint, flour, pop, and sweet corn. Sweet corn (*Zea mays saccharata* Sturt) is planted mainly for human consumption and is characterized by the presence of one or more recessive alleles such as *sugary1* (*su1*) and *shrunken 2* (sh_2) that alters the carbohydrate content in the endosperm of the corn in favour of high sugar (Revilla et al., 2021), hence the sweetness that is favourable for human consumption.

Cultivation of sweet corn is increasing in Malaysia from 6,590 hectares in 2003. to 9,761 hectares in 2023 corresponding to an increase in production from 31,907 in 2003 to 67,891 metric tonnes in 2023 (Department of Agriculture, 2023). The monetary value in 2021 alone was 209 million Malaysian Ringgits (DOA, 2022). Despite the surging demand for fresh consumption and processed sweet corn, most of the local seed requirement is mainly imported especially from Thailand and Taiwan (Sa'at et al., 2012). Although, Malaysia through the government and private entities had released many varieties of sweet corn (such as Masmadu, Sweet Pacific, and Hibrimas), imported sweet corn hybrid seeds are more popular among the farmers due to their higher yield and market potential overshadowing the local hybrids. Total import of

seeds has been increasing over the years and this results in a deficit in the Malaysian Balance of Trade (BOT), hence a plan is needed to strengthen the nation's seed production programme (Suhana et al., 2017). The above scenario has resulted in private companies such as Green World Genetics Sdn. Bhd., to focus on hybrid seed production leading to the release of a *shrunken2* sweet corn commercial hybrid, with the codename GSH11005Y.

The development of any new sweet corn hybrids must be accompanied with good quality seeds for mass cultivation. However, a major challenge in sweet corn seed production is seed storability. Generally sweet corn seeds have poor storability and lose both viability and vigour in a relatively short of time after harvest for example in a korean sweet corn, 'Hybrid Honey 236' exhibited a decline in germination from 84% to 52% after a year of storage as opposed to other types of corn (Chiu et al., 2003). This low storage potential causes low and erratic germination, poor seed vigour and deprived seedling establishment (Pairochteerakul et al., 2018; Styer & Cantliffe, 1983). Several research to identify the root cause of poor storability in sweet corn seed, attributed it to the presence of mutant gene in sweet corn kernel (Borowski et al., 1991; Pairochteerakul et al., 2018; Parera et al., 1998; Suo et al., 2017; Tracy, 2000; Wilson & Mohan, 1998).

The sweetness in sweet corn is achieved with the presence of several mutants such as *shrunken2* which reduces the starch biosynthesis. These mutants work by interrupting the production of several enzymes responsible in sugar to

starch conversion, maintaining a high sucrose content (Tracy, 2000). This reduction of starch content in the kernel of sweet corn, however, has a positive correlation with poor germination, vigour and its storability(Douglass et al., 1993; Soriano et al., 2011). Starch is an important seed reserve which is mobilized during seed germination (Hao et al., 2018) as one of primary source of carbohydrate in endosperm to fuel embryonic axis emergence upon imbibition (Shaik et al., 2014). He & Burris (1992) stated that most of the *shrunken2* sweet corn would exhaust its starch content, four days in germination. Low starch content in sweet corn seed also resulted in poor structural integrity during post-harvest drying because the process would shrink the endosperm of sweet corn, resulting in air pockets in between endosperm and pericarp of sweet corn, making it susceptible to physical damage such as cracking thus reducing seed storability(Gupta et al., 2005). Despite a correlation between poor quality of sweet corn with reduced starch, sucrose content on the other hand is not directly related to seed viability.

Other than genetic factor, the time when seeds are harvested is another important factor in determining seed quality and currently, the suitable time to harvest sweet corn seed is varied among varieties. For example, 'Supersweet17', a *shrunken2* sweet corn seed is harvested between 17 to 30 days after pollination (DAP) while 'Chaotian 2018', also a *shrunken2* sweet corn is best harvested at 38 DAP. Optimal harvest time must be identified for GSH11005Y to ensure only matured seed is harvested and the period of

harvest must not be too late to avoid damage by environmental condition such as pathogen attack or field weathering.

After harvest, seeds are usually further dried until it reaches the desirable moisture content of around 8 to 10% for storage purposes (Darrah et al., 2019). The drying temperature varies among the seed producers with no clear recommendations. Scholars working on the effect of varying drying temperatures ranging between 35°C to 60°C reported that different temperature had different effects on seed quality of sweet corn (Navratil & Burris, 1984; Herter & Burris, 1989; Guiscem et al., 2002; Gupta et al., 2005).

When the sweet corn is harvested for human consumption, sweet corn cob is harvested earlier and will be kept at lower temperature to maintain its sucrose content. Many studies have shown that sucrose content decreased when the sweet corn kernel is subjected to high temperatures and at the same time would result in an increment of starch content (Tracy, 2000). Considering this insight, one could wonder whether the sucrose and starch content in sweet corn seeds could be influenced by the drying temperature, and if implementing such an approach in seed production would result in enhanced seed quality and longevity.

Other than the initial seed quality during harvest, for long-term storage, post-harvest environmental condition during storage period also affects seed viability. Two factors that have been widely studied to improve seed

storability especially for commercial purposes, are seed moisture content and the temperature during storage. On the other hand, one of the factors that has been overlooked is the oxygen availability during storage. Oxygen is important as it regulates a series of physiological processes through respiration (Rajjou & Debeaujon, 2008) While oxygen is important, it also leads to seed deterioration as it regulates a series of activities which result in the accumulation of reactive oxygen species (ROS) such as superoxide and hydroxyl that has detrimental effect on seed longevity (Wang et al., 2018). Commercially, sweet corn seeds are commonly stored in air-tight sealed packaging. In other crops, such as sunflower (Abreu et al., 2013) and rye (Barzali et al., 2005), studies have shown that the use of vacuum packaging in which seeds are sealed in very low oxygen level by evacuating air from the package, improves seed longevity. However, this has never been demonstrated in sweet corn seed.

As much as we can prolong longevity, deterioration process is inevitable and results in reduced seed germination and vigour. Hence, there is a need to develop a method to improve seed quality of aged or deteriorated seeds to maximize seed potential so that every seed can be grown into a functional plant. Seed priming is one of the methods that has been widely used to enhance seed germination as well as its vigour. This method involves a pre-sowing procedure by controlling hydration through seed imbibition using natural or synthetic compound and re-drying, which allows a more rapid germination when the seeds are re-imbibed (Zulfiqar, 2021). Several types of

priming exist such as hydropriming, halopriming, osmopriming, solid matrix priming and others (Sher et al., 2019). Today, the use of hydrogen peroxide (H_2O_2) as pre-treatment to improve plant growth is getting much attention. H_2O_2 is a reactive molecule that plays an important role in plant, but it is also responsible for seed deterioration. However, at certain concentration, H_2O_2 priming can enhance abiotic stress tolerance by modulating ROS detoxification and by regulating multiple stress-responsive pathways and gene expression (Hossain et al., 2015), therefore it has a potential as osmopriming agent to revive seed quality.

Realising the above information, a study was conducted with the aim to improve germination and storability of sweet corn seed based on the following objectives:

1. To identify the optimum harvest maturity stages and efficient drying temperature to improve seed quality and storability of sweet corn seeds,
2. To determine the effects of vacuum packaging and storage at different temperature on the longevity and quality of sweet corn seeds,
3. To study the potential of hydrogen peroxide as priming agent to reinvigorate stored sweet corn seeds.

CHAPTER 2

LITERATURE REVIEW

2.1 Sweet Corn in Malaysia

Corn is among the top three agricultural crops grown globally after rice and wheat (Erenstein et al., 2022). It can be grown under various climates making it very popular worldwide with a production of 1,210,235,135 thousand tonnes in 2021 alone (FAO, 2023). Corn is a staple food for many populations especially in United State of America (383,943 metric tonnes per year) as well as Asian countries such as China (272,762 metric tonnes per year) and is not limited to human consumption but also for animal feed and other industrial purposes. Among the types of corn grown, sweet corn is favoured for human consumption due to its high sucrose content.

It has been no secret that the demand for sweet corn in Malaysia is increasing over the years. Statistically, the area planted with sweet corn has increased over the years (9,761 hectares in 2022) with a total production of 67,891 metric tonnes in 2022 alone (DOA, 2023). The Department of Statistics (2021) also showed an increase in the per capita consumption (PCC) index of sweet corn demand from 1.5 kg /year in 2019 to 1.7 kg /year in 2020. Currently, Malaysia's self-sufficiently ratio (SSR) of sweet corn has exceeded 100% (105.6%), however, the local seed requirement is depended on imports from

Thailand and Taiwan (Sa'at et al., 2012). To improve this for food security reason and for seed security, several companies in Malaysia are exploring the potential of producing hybrid seeds.

Green World Genetics (GWG) Sdn. Bhd. is one of the Malaysian companies focusing on the production of sweet corn seeds, for both local and international market trade. In 2019, the company released a new variety of sweet corn, code-named GSH11005Y which is a yellow type of sweet corn seed with high kernel number and sucrose content compared to other yellow type sweet corn produced by Green World Genetics (GWG) Sdn. Bhd.

2.1.1 Plant Morphology of Sweet Corn

Sweet corn belongs to the grass family of *Poaceae*. The plant can grow from 1.5 to 3 meters in height with a cylindrical or solid pith which is divided into nodes and internodes. The number of internodes vary from 14 to 20 depending on the variety. Each plant usually has at least eight to 20 leaves that are arranged spirally on the stem and they occur alternately in two opposite rows on the stem. The leaf consists of sheath, ligules, auricles, and a blade. The leaf blade is long, narrow, has undulating margin and tapers towards the tip. The plant has a branched fine fibrous root system. The root extends 1.5 meters laterally. It has adventitious roots that develop in a crown of roots from nodes below the soil surface (Hallauer, 2000).

The inflorescence of sweet corn consists of both male and female flower on the same plant, botanically termed as monoecious. The male flower is borne in the tassel while the female flower grows on the ear. The female flower bears the silk that are receptive to pollens for approximately three weeks. The female flowers will develop into young cobs, upon fertilization. The cob has the pistillate spikelet which contains ovary, style, and stigma and ovules ranging from 300 to 1,000 depending on the variety (Paliwal et al., 2000).

The flowers can be crossed or self-pollinated and is often wind pollinated. The fruit of sweet corn is known as sweet corn kernel, a caryopsis type of fruit (Hallauer, 2000). Sweet corn kernel can be divided into seed coat (pericarp), a diploid embryo (germ) and a triploid endosperm (Rivera-Castro et al., 2020) as depicted in 2.1.

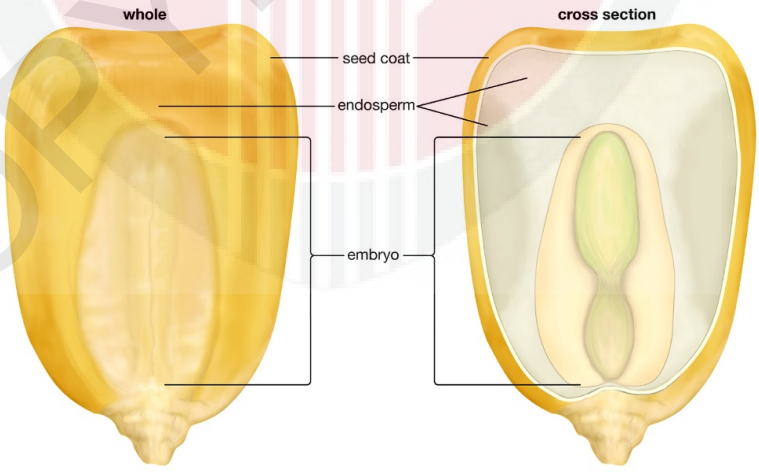

Figure 2.1: The cross section of sweet corn kernel showing the seed coat, endosperm, and embryo of sweet corn. Adapted from Encyclopaedia Britannica (2013).

2.1.2 Composition of Corn Kernel

Corn kernel consists of endosperm that is 83% of dry weight, followed by, embryo (germ) at 11% and a pericarp (seed coat) weighing around 6% of kernel dry weight. Corn kernel comprises of carbohydrate, protein and lipid and other micronutrients such as fibre, vitamins, and minerals (Nuss & Tanumihardjo, 2010). Generally, the type of carbohydrate in most corns are starch, totalling around 72-80% of kernel dry weight while sugars only amounting between 1 to 3% with sucrose as the most abundant followed by glucose, maltose, fructose and raffinose in a trivial fraction (Boyer & Hannah, 2000).

While the type of carbohydrate in most corns is starch, the sweet corn is unique with its high level of sweetness compared to the rest, attributed to the amount of sugar in the form of sucrose in the endosperm (Tracy et al., 2019). Table 2.1 below shows the carbohydrate component in different types of corn collected at different kernel age. Corn with *shrunken2* gene had the lowest amount of starch ranging between 18.4 to 22.3% but had 83% more sucrose than dent corn at 20 DAP (harvest maturity for human consumption).

Table 2.1: Carbohydrate composition in endosperm of dent corn in comparison with s*ugary* and s*hrunken*2 sweet corn. Adapted from Tracy (2000).

Genotype	Kernel Age (DAP)	Carbohydrate Composition in Endosperm (%)			Total Carbohydrate Content of Kernel (%)	Max Seed Dry Weight (mg)
		Starch	*Sucrose*	*Reducing Sugar*		
Dent Corn (*Normal*)	16	39.2	17.6	3.7	60.5	246
	20	66.2	5.9	2.8	74.9	
	24	69.2	4.8	2.8	76.1	
	28	73.4	3.0	2.2	78.6	
Sweet Corn (s*ugary*)	16	23.3	25.7	14.3	65.3	134
	20	28.0	15.6	22.8	66.5	
	24	29.2	13.1	28.5	70.8	
	28	35.4	8.3	24.2	69.6	
Sweet Corn (s*hrunken 2*)	16	22.3	28.3	5.6	56.1	74
	20	18.4	34.8	4.4	57.6	
	24	19.6	29.4	2.4	51.4	
	28	21.9	25.7	5.1	52.8	

2.1.3 Carbohydrate Synthesis in Sweet Corn Kernel

To understand the reason behind the difference between carbohydrate composition of sweet corn and normal corn, a comprehension on starch synthesis is needed. Once sucrose enters cytosol, it will be converted into fructose and UDP-glucose and eventually to a glucose 1-phosphate by sucrose synthase. Another enzymes known as ADP-glucose pyrophosphorylase (AGPase) would catalyse the conversion of glucose 1-phosphate into ADP-Glucose before transporting it to plastid amyloplasts. Three types of enzymes, starch synthase, starch branching enzymes and debranching enzymes will catalyse the ADP-glucose into crystalline starch granules (Smith, 2001; Tracy et al., 2019). The process is illustrated in Figure 2.2.

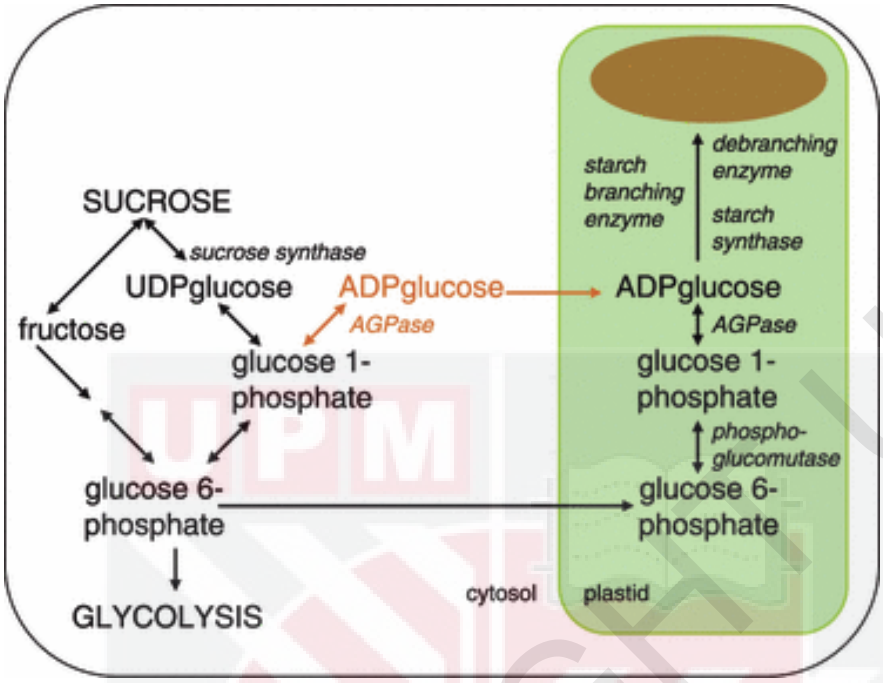

Figure 2.2: Overview of sucrose to starch conversion during starch synthesis in storage organ such as endosperm of corn. Adapted from Smith (2008).

2.1.4 Mutant Effect on Carbohydrate Synthesis in Sweet Corn Kernel

According to Coe et al. (1988), sweet corn has a genetic modification in its endosperm by mutant genes which interrupt the conversion of sucrose to starch. To date, fourteen mutants have been studied, with eight being commercially used and it works by interrupting the starch synthesis (Tracy et al., 2019). These starch synthesis mutants can be divided into two; class 1 mutant such as *brittle1* (*bt1*), *brittle2* (*bt2*), and *shrunken2* (*sh2*) which accumulates sugar at the expense of starch by interrupting the production of certain enzymes needed in sucrose to starch conversion (Whitt et al., 2002). For example, in *shrunken2* (*sh2*) the production of ADP-Glucose

pyrophosphorylase (AGPase) is reduced up to 90%, which disables the seed to store carbohydrate in the form of starch, leaving seeds to be high of sucrose content in the kernel (Hossain et al., 2019). The role of this gene is illustrated in Figure 2.3.

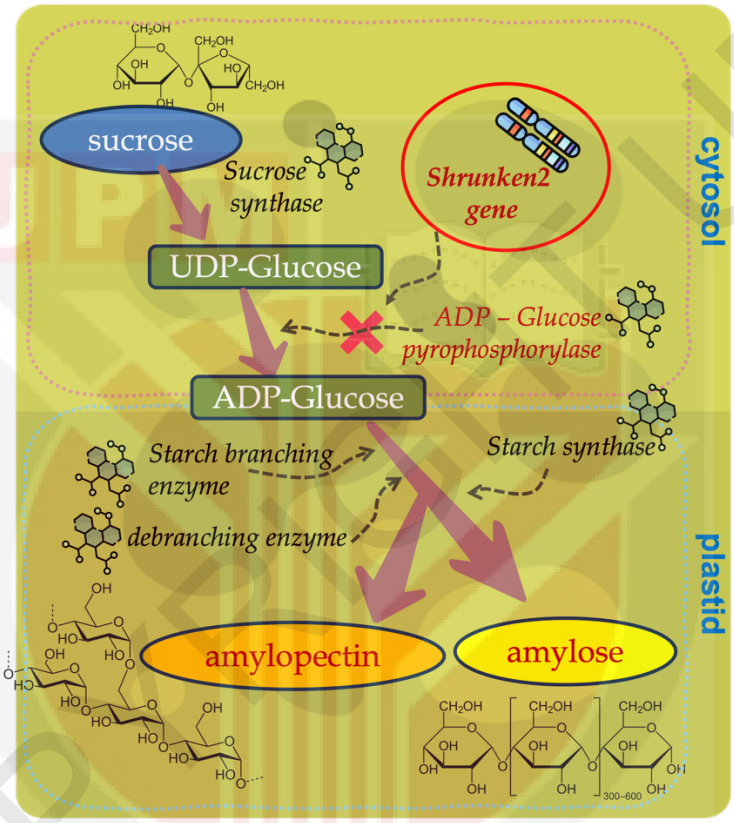

Figure 2.3: Five types of enzymes (sucrose synthase, ADP-Glucose pyrophosphorylase, starch branching enzyme, debranching enzyme and starch synthase) are involved in the conversion of sucrose to starch. The *shrunken2* gene inhibits the production of ADP-Glucose pyrophosphorylase resulting in poor conversion of sucrose to starch in sweet corn endosperm.

On the other hand, class 2 mutants such as *amylose extender1* (*ae1*), *dull1* (*du1*), *su1*, and *waxy1* (*wx1*) alter the types and number of polysaccharides produced to maintain the simple saccharides, resulting in higher water-soluble polysaccharides, or also known as water-soluble sugar. Both classes, result in the sweetness when kernels are consumed (Hallauer, 2000).

2.1.5 Carbohydrate Content and Seed Quality of Sweet Corn

While the higher sucrose content improves the eating quality of corn, it also affects seed quality especially germination, storability, and field emergence. High sucrose content especially in *sh2* corn, results in very slow drying due to the osmotic potential. This slower rate facilitates the seed-borne pathogenic incidence such as *Fusarium* spp. which eventually reduces the germination and vigour of sweet corn seed (Wilson et al., 1994).

Improving sucrose content also means there will be a low starch concentration in *sh2* corn. This results in severe shrinking of the endosperm as it dries, and it creates several structural problems for the seed. The shrinking endosperm pulls away from the pericarp, creating air pockets between endosperm and the pericarp. Air pockets increase the kernels' susceptibility to physical damage during handling and can result in the cracking of the pericarp. The pericarp itself acts as an impediment to pathogens and water movement, both into and out of the kernel. A damaged pericarp greatly reduces germination in all endosperm types (Gupta et al., 2005).

According to Tracy et al. (2019), the seed weight of *sh2* corn is 44.77% lesser compared to that of *su1* corn and 70% lesser than dent corn (Table 2.1) and seed weight is usually correlated to germination capability. Diminution in sweet corn seed weight is essentially due to greatly reduced starch levels

relative to other types of corn, hence the shrunken appearance of sweet corn seeds. Consequently, starch levels can affect germination and seed vigour of sweet corn especially for *sh2* sweet corn varieties.

Seeds are a mean of survival, and it requires long term carbon stores to fuel seedling establishment. This storage compound came from the partitioning of assimilated carbon (usually in the form of sucrose) before being converted into inert and water insoluble compound, i.e., starch. In corn, the seed reserve in the form of starch is deposited into its endosperm (Tetlow, 2011). Once favourable condition for germination is present, the scutellum would synthesize gibberellin, which then secretes alpha amylase in aleurone layer to hydrolyse starch into simpler sugar to fuel the germination activity (Yan et al., 2014). According to Soriano et al. (2011), starch also is positively correlated to seed viability in many crops where its reduction will affect seed quality (Zhao et al., 2018).

It is therefore, essential to conclude that the carbohydrate changes especially starch and sucrose content is important in determining the objectives of harvest and this also relates to the time to harvest the sweet corn kernel, either for human consumption or seed production. Harvesting sweet corn kernel for seed production at the optimum maturity time will not only provide the highest quality, but also improve the seed storability, a prominent problem observed in most sweet corn varieties.

2.1.6 Kernel Development and Harvest Maturity for Human Consumption and Seed Production

Carbohydrate changes can be observed during sweet corn kernel development. Sucrose is transported from leaves to internodes before ending up in developing kernel via phloem and this happens once fertilization takes place (Hütsch & Schubert, 2017). Sucrose content is usually the highest during immature milky stage, that is between 18 - 22 DAP and typically harvested at this stage of maturity for human consumption (Lertrat & Pulam, 2007; Szymanek et al., 2015).

Dr. Pek's 1351, an F_1 *sh2* corn from Thailand showed an increase in sucrose during kernel development after pollination which peaked at 18 DAP and gradually reduced while the starch content increased before stagnating at 38 DAP. The maximum 100 seed dry weight was observed at 35 DAP indicating that Physiological Maturity (PM) was at 35 DAP (Harakotr et al., 2022).

Cao et al. (2008) studied the changes of kernel nutritive components (sugar, starch, and protein) during kernel development of Shuxuan 1, a F_1 *sh2* sweet corn variety. In the study, both sucrose and starch increased with maximum sucrose content at 21 DAP before it decreased while starch content peaked at 30 DAP before stagnating.

From the information above, harvesting time for different sweet corn varieties were varied. In other studies, different harvest maturity index was used, for example, Lee et al. (2004) used days after silking (DAS) to indicate the level of maturity in a study using Korean sweet corn varieties namely Hybrid Early Sunglow, Golden Cross Bantam 70, Xtrasweet 82 and Fortune. It was concluded that it was best harvested between 42 to 49 DAS. The stage of maturity of DAS is based on the silking date where female anthesis has taking place.

Some studies used days after pollination (DAP) or days after anthesis (DAA) to indicate the maturity stages in which seeds can be harvested. DAA is conceptualized based on the time when tassel start shedding pollen, henceforth, a male anthesis. Chaotian 2018, Chaotian No. 3 and Shuyu No. 1 are varieties of *sh2* sweet corn from Hangzhou Vegetable Science Institute, China and were best harvested at 38 DAP with the highest germination, and vigour qualities in a study by Guan et al. (2013). On the other hand, Supersweet 17, also a *sh2* sweet corn variety from China can be harvested at 28 to 30 DAP (Cao et al., 2010) while Supersweet 18 and Shuxuan No. 1 had the best seed quality when harvested at 38 DAP although maximum starch content was observed as early as 30 DAP (Cao et al., 2008). In Thailand, 101LTSC-10, TSC/H3-7and P4546/Delec-1 sweet corn was best collected at 38 DAP while 'C4' had the best quality when harvested at 42 DAP (Harakotr et al., 2022).

Apart from time of harvest, seed moisture at harvest also plays an important role in determining seed quality. For instance, in a study by Bennett et al. (1988), it showed that sweet corn seeds harvested at 45% to 54% moisture content before post harvesting drying had superior stand establishment compared to the seeds that had moisture of 35%. Gupta et al. (2005) also harvested seeds at two different moisture content which was at 20-30% and 40-50% (both further dried into optimum harvest moisture content) with seeds harvested at higher moisture having better germination either using standard germination and cold germination.

Hence, harvesting at right time in which seed is at maximum level of starch and lowest starch content within the right moisture may improve seed quality especially for sweet corn storability.

2.1.7 Post-Harvest Drying for Sweet Corn Seed Production

Sweet corn cob is usually harvested between 50 to 55% of moisture content (George et al., 2003), followed by further drying to around 8 to 10% for storage (Hallauer, 2000). It is known that high moisture content during storage will lead to seed deterioration thus reducing the seed quality (Bewley et al., 2013), hence the need for drying. Effects of drying temperature on sweet corn seed quality has been studied by many scholars such as Navratil & Burris (1984), Herter & Burris (1989), Guiscem et al. (2002) and Gupta et al. (2005), with drying temperatures ranging from 30°C to 60°C.

Sweet corn seeds that were harvested at 38% to 48% moisture content can be dried at 50°C based on the study by Navratil & Burris (1984) while according to Guiscem et al. (2002), drying of seeds at 60°C could be fatal to the seed viability. However, a study by Gupta et al. (2005) on the effects of drying temperature at 30°C, 40°C and 50°C showed that drying at 50°C is detrimental causing massive reduction in seed germination after 18 months after storage (MAS). While no significant difference was observed between 30°C and 40°C during the initial storage, seeds dried at 40°C had better germination after 18 months. However, there was no further studies conducted to identify the root cause for this phenomenon. Currently, in industry practices such as GWG Sdn. Bhd., it practices drying at 35°C to 38°C.

2.1.8 Changes in Kernel Carbohydrate Content During Post-Harvest Handling

As these mutants in sweet corn affect the carbohydrate composition in the endosperm, changes also can occur due to post-harvest conditions. When sweet corn kernel is stored at room temperature (27°C), the endosperm would elude 50% or more of its sucrose content after 24 hours (Hallauer, 2000). However, the loss of sucrose can be reduced by cooling the freshly harvested corn cobs (Boyer & Shannon, 1983) and to understand this, Table 2.2 below is referred.

In a study by Garwood et al. (1976), a reduction in sucrose content was observed when the *sh2* sweet corn kernel was placed at a higher temperature compared to low temperature. In contrast, starch content increased after the sweet corn was placed at high temperature of 27°C (room temperature), from 2.9% to 9.7% after four days. Hence, for human consumption, sweet corn is currently stored at lower temperature to retain the sucrose content and its sweetness.

Table 2.2: Carbohydrate content changes for the *sh2* sweet corn after one, two and four days in storage at 4°C and 27°C. Adapted from Garwood et al. (1976).

Storage Condition		Carbohydrate (% of dry weight basis)		
Time (hours)	Temperature (°C)	Reducing Sugar	Sucrose	Starch
0 (initial)	-	5.3d	36.5a	2.9b
24	4	7.0abc	32.7b	5.0ab
48	4	7.9a	30.6b	5.9a
96	4	7.6ab	33.ab	5.6a
24	27	5.7cd	29.0b	5.2a
48	27	6.0cd	28.2b	8.3a
96	27	4.4d	13.5c	9.7a

A study by Olsen et al. (1990) also showed the same result where three commercial sweet corn varieties, Aussie Gold 12, Rosella 425 and Sucro, had their sucrose content reduced when stored at 18°C in comparison to storage at 1°C and 4°C, with the increase of starch content at 7°C and 18°C.

Apart from sweet corn, waxy corn which is famously consumed in several countries in Asia shows the same trend. Two popular waxy corn in Thailand,

Tien Baan Kao and Big White 852 had a rapid loss of sucrose when stored at ambient temperature, whilst the total starch, amylopectin and phytoglycogen were upsurged (Simla et al., 2010).

However, these studies were limited to sweet corn for human consumption, which notably is harvested at a much earlier stage compared with that for seed production. Unfortunately to date, there is no study on how these changes in temperature affects the drying process and its carbohydrate content for sweet corn seed production. Thus, it raises the question if the sweet corn kernel is dried at a much higher temperature as being practiced currently in sweet corn seed production, will it also affect the sucrose and starch level in sweet corn and result in enhanced seed quality and longevity such as observed in the study by Gupta et al. (2005) in Section 2.1.7.

2.2 Seed Storage

Many seeds are capable of surviving dehydration at maturity, which prolongs longevity. However, deteriorative chemical processes continue in dry seeds, resulting in gradual loss of vigour and eventual death (Bewley et al., 2013). While various factors can influence seed longevity, the two most important ones are seed moisture content and temperature (together with relative humidity) (McDonald & Copeland, 2001). Harrington's Rule states that storage life will approximately double for each 5°C decrease in temperature and each 1% decrease in seed moisture content for temperatures between 0

and 40°C and moisture contents between 5 and 14%. Both rules emphasize the importance of low seed moisture content and temperature in extending the longevity of seeds, and to avoid the combination of high temperature and moisture content simultaneously (Harrington, 1972).

2.2.1 Factors Influencing Seed Deterioration During Storage

Various factors have been identified to influence seed ageing in storage, with the most common and widely discussed factor being the temperature and seed moisture content. Recent studies such as by Wang et al. (2018) on rice, Mbofung et al. (2013) on soybean and Thirusendura & Saraswathy (2018) on onion showed that high temperature promotes many enzymatic and metabolic activities while high humidity will increase the rate of hydrolytic, enhance respiration as well as free fatty acid.

Bewley et al. (2013) stated that a consistent relationship existed between temperature, relative humidity, and moisture content on seed ageing. An increment in relative humidity can caused an increment in moisture content of the seed which in turn results in increased seed ageing (Rao et al., 2017). Although moisture is reduced, high temperature also may fasten the seed ageing process as it induces chemical reactions related to seed ageing (Zhang et al., 2021). McDonald & Copeland (2001) suggested that for seeds stored between 4°C to 10°C, the humidity should be no higher than 70% to slow down the ageing process. Harrington (1972) stated that seed viability can be

maintained if the moisture content of the seed is between 5 to 14% while Jyoti & Malik (2013) stated that the optimal moisture content that will prolong seed longevity is around 6 to 8%.

While temperature, relative humidity and moisture content are the most important conditions affecting seed longevity in storage, several other factors also contribute to seed longevity. Oxygen in particular, influences seed deterioration via oxidative reactions (Rajjou & Debeaujon, 2008). There is consensus that oxidative and peroxidative processes play a primary role in initiating the damage that occurs in dry seeds (Gerna et al., 2022; Jeevan et al., 2015; Zhang et al., 2021).

Free radicals can be generated spontaneously and can trigger oxidation of various seed constituents. The availability of oxygen and its tendency to form reactive oxygen species (ROS), including H_2O_2 and hydroxyl radical (OH^-) allow free radical reactions to occur, albeit at slower rates (Huang et al., 2019). On the other hand, the presence of water molecules tends to quench free radical mechanisms and enable antioxidant mechanisms to be ineffective. Thus, damage due to free radicals and ROS is most prevalent in wet seeds (Guan et al., 2009; Wang et al., 2018).

ROS has been accepted to have a dual role in seed physiology especially in a dormant seed. H_2O_2 is high during seed development and decreases as seeds attain desiccation tolerance. During imbibition, it was observed that H_2O_2

increased due to respiration in mitochondria. This resulted in superoxide (O_2) and H_2O_2 production, and the accumulation of this works as a signal of germination in regulation of ROS producing and scavenging mechanism (Bailly et al., 2008). ROS also plays an important role in alleviation of dormancy as well as mechanism to counter pathogens (Waszczak et al., 2018). However, ROS accumulation in stored seeds also acts as a first step associated with seed ageing process.

It is known that an imbalance of ROS in seeds causes oxidative damage which leads to poor viability as can be seen in aged seeds. In aged seeds, it was observed that the accumulation of ROS such as O_2^-, H_2O_2, OH^- and singlet oxygen ($^1O_2$) among many others that causes many damaging activities such as lipid peroxidation, chromosome aberration, protein carbonylation, damage to membrane cell and so on (Sharma et al., 2012).

According to Sano et al. (2016), peroxidation of lipids, initiated by the abstraction of a hydrogen by OH^-, can result in a chain reaction that causes breakdown of the lipids (particularly unsaturated lipids) and release of by-products such as reactive aldehydes that can cause further damage to proteins and nucleic acids. Changes in membrane lipids due to peroxidation involved in the increase in membrane permeability and cellular leakage that is associated with seed aging. Reactive carbonyl by-products of peroxidation can also result in Amadori products, which are sugar-protein complexes and intermediates in the Maillard reaction that result in the nonenzymatic

oxidation of biological molecules. Protein modifications such as nonenzymic glycosylation by reducing sugars can alter their structural integrity and inactivate enzymes that are required during early imbibition. ROS can also damage DNA, and chromosomal abnormalities and increase in frequency as seeds age (Groot et al., 2012).

However, antioxidants, such as tocopherols, phenols, ascorbate, and antioxidant enzymes can destroy these free radicals and ROS and stop their propagation, reducing damage to the seed. The mechanisms to regenerate these antioxidants eventually will be inoperative, so it is expected that they would ultimately be exhausted, enabling ROS activity to become further damaging (Wilson & McDonald, 1986).

2.2.2 Changes during Seed Deterioration

There are many events occurring during the seed ageing process including lipid peroxidation, alteration of enzyme activities, DNA damage, chromosome aberration among many others (Zhang et al., 2021).

One regular observation that can be made in the aged seed is the increase in seed leachates when it is soaked in water as a reflection of membrane damage (Copeland & McDonald, 2001). Based on the review by Zhang et al. (2021), increase in leachates can be attributed to the phospholipid's bilayer of the seed plasma membrane. As the deterioration occurs, the shape of this

phospholipids layer shifted from cylindrical shape to cone making it unable to maintain its double layer, thus losing its selective permeability allowing leachates to come out when seeds are soaked in water. This leachate can be measured using the electrical conductance method (Abdul-Baki & Anderson, 1970). The degree of damaged can be identified based on the concentration of the exudates themselves (Zhang et al., 2021). For example, the higher release of Ca^{2+} in leachates is a sign that membrane structure had been compromised due to excessive accumulation of Ca^{2+} in the seeds. The accumulation of it also led to mitochondrial dysfunction which leads to ROS accumulation, thus causing ageing in seed (Li et al., 2017). Lipid peroxidation also can lead to membrane damage and increases the leachates as it forms hydroperoxides as the end product of oxidation of unsaturated fatty acid, thus damaging membrane permeability (Ebone et al., 2019).

Apart from measuring the seed leachate using electrical conductance method, lipid peroxidation also can be measured based on its by-product, malondialdehyde (MDA). It is considered as the main biomarker to measure the extent of damage by oxidation, and it is also an end product of lipid peroxidation. A study by Min et al. (2017) on controlled deterioration in soybean showed that higher MDA and H_2O_2 was observed compared to control seeds.

Seed ageing often also means loss in enzyme activities and can be used in measuring seed deterioration (Wang et al., 2015). During seed ageing, many

important enzyme activities, and function in maintaining its quality are decreasing. This includes the ATP synthesis, amylase, protease, lipoxygenase among many others (Zhang et al., 2021). As deterioration occurs, ROS accumulates, causes depletion in antioxidant enzymes which further damages the seeds. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and glutathione peroxidase (GPX) play an important role in combating the damage by ROS, and reduction can be an indicator in aged seeds (Bailly et al., 2008). Ebone et al. (2019) stated that the first occurrence of deterioration in the seed can be attributed to the depression of these antioxidant activities due to enzyme inactivation.

2.2.3 Antioxidant Enzymes in Seed Deterioration

There are several antioxidant enzymes responsible for combating seed deterioration due to ROS as to reduce the seed ageing process. SOD, CAT, ascorbate peroxidase (APX), glutathione peroxidase (GPX), guaiacol peroxidase (POD), peroxiredoxin (PRX), monodehydroascorbate reductase (MDHAR), dehydroascorbatereductase (DHAR) and glutathione reductase (GR) act as the protective mechanism developed by seed to counter back or neutralize the ROS to avoid further damage. Apart from that, there are a few non-enzymatic mechanisms that also protect the seed from ROS damage such as ascorbic acid, tocopherols, flavonoids, carotenes among many others (Zhang et al., 2021). The activity of these protective mechanism can be summarized into Figure 2.4.

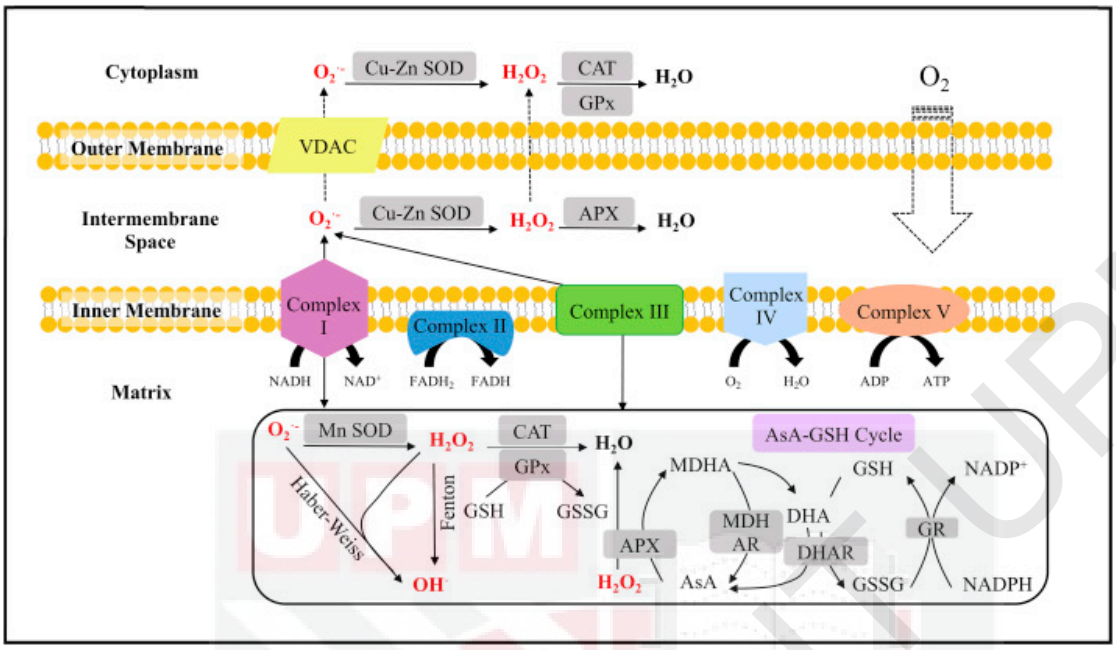

Figure 2.4: The activity of antioxidant as protective mechanism against ROS. Adapted from Zhang et al. (2021).

Reduction of these mechanism particularly the antioxidant enzymes is responsible to the accumulation of ROS, leading to seed ageing. A study by Xin et al. (2014) showed than the level of superoxide in imbibed rice increased up to 58% after one MAS and 123% more after nine MAS. It was shown that enzymatic activities such as CAT and POD dropped during the period. It was also observed that SOD in mitochondria also was exhausted, leading to higher ROS accumulation. Other crops such as oat (Kong et al., 2015) also showed a great reduction in enzymatic activities. Eventually, depression during these activities lead to excessive accumulation of ROS leading to many damaging events such as lipid peroxidation, protein carbonylation, genetic damage and so on.

Based on our understanding on the above topic, seed storage should be planned accordingly so that seed life shell can be prolonged. Other than moisture content and temperature, the oxygen is one of the key factors that affect seed deterioration and loss of viability (De Vitis et al., 2020). Therefore, seed storage environment (temperature and relative humidity) and suitable packaging to store sweet corn seed should be based on this understanding.

2.2.4 Storage Temperature

The temperature of the environment in which seeds are stored play an important role in determining how long seeds viability can be retained. Usually, seeds can be stored in either ambient room temperature or cool temperature with the latter being preferred to prolong seed viability. Generally, effects of temperature during storage have been reviewed in Section 2.2.1 earlier. However, the ideal temperature for seed storage is different depending on the species. For example, for peanut seed, the suitable temperature is at 10°C (Morton et al., 2008), while for amaranth it is 16°C (Aufhammer et al., 1998).

A study conducted by Tathiana & Marcos-Filho (2013) on how environmental temperature affects corn seed after 15 MAS under three different environments, cold and dry environment (Temperature: 10°C, RH: 30%), controlled environment (Temperature: 20°C, RH: 50%), and lab environment (Temperature: 14-31°C, RH: 72-83%), showed that seed stored in cold and dry

environment (low moisture and low humidity) prolonged seed viability while controlled temperature had lower viability after 15 months.

Apart from the above, many studies exist on the storage of sweet corn seed using different temperatures. Gupta et al. (2005) used 30°C to store sweet corn seed while Chiu et al. (2002) stored sweet corn seed at 25°C. Lee (2001) on the other hand, stored the sweet corn seed at room temperature (ranging between 25-30°C) to learn different seed deterioration responses of sweet corn based on the types of mutant. A comparison study of ambient room temperature (Temperature: 25°C, RH: 70%) with cold room temperature (Temperature: 16°C, RH: 60%) and how it affects seed physiological performance was conducted by Deuner et al. (2014) and found that storing corn seed at cool room had better viability and vigour.

2.3 Seed Packaging

According to Rao et al. (2006), storing seeds in hermetically sealed packaging, the use of desiccants as well controlling temperatures can help in prolonging seed storability as physiological and biochemical activities discussed and reviewed in Section 2.2 can be regulated in dry packaging storage.

There are several studies conducted on seed packaging types and how it affects seed storability. According to Naguib et al. (2011), the best packaging type for wheat was aluminium and polyethylene (PE) bag which maintained

the viability of more than 85% even after 18 months in storage of five wheat varieties. Polyethylene (PE) bag showed promising result when it comes to prolonging seed viability. This can be seen in the medicinal plant such as *Abelmoschus moschatus* (Tiwari et al., 2022) and oily seed such as *Arachis hypogea* (Fu et al., 2018). Modified PE such as metalized PE (aluminium PE) also had been used in cereal seeds such as *Oryza sativa* (Alahakoon et al., 2021), and legumes like *Gylcine max* (Arief et al., 2022; Chuansin et al., 2006).

A study by Coradi et al. (2020) on how packaging technology can affect soybean seed viability under different storage temperature showed that soybean stored in polyethylene coated with raffia packaging in natural room temperature environment also had same rate of deterioration with those stored in cool temperature. This showed that under right packaging type, seeds are capable of storage in normal temperature without losing its viability rapidly, proving an excellent alternative when storing in cool temperature is inaccessible.

2.3.1 Sweet Corn Seed Packaging

Most sweet corn seeds are packed in air-tight sealed PE or metalized PE packaging such as in Figure 2.5. Moisture content, viability and vigour based on cold test showed that the seeds stored in hermetic condition in PE packaging performed well compared to aluminium packaging, paper, and

raffia packaging as long as it is under hermetic condition to control oxygen level within the package (Capilheira et al., 2020)

Figure 2.5: Sweet corn seed are packed inside metalized polyethylene bag that is air-tight sealed.

2.3.2 Vacuum Seed Packaging

At the moment, the oxygen level was only controlled by using a hermetically either in the hermetic container or inside PE packaging for example in rice (Alahakoon et al., 2021). Despite the influence of the oxygen level during seed storage on seed deterioration, it has hardly been considered in the practice of commercial packaging or gene-bank seed storage (Groot et al., 2012).

Vacuum packaging is a technology where the product is placed or packed in a suitable material and oxygen is drawn out by the mean of removal of air before sealing the package (Rooney, 1995). These method has been used in packaging processed food products such as snack food, tea, coffee among many others (Chandra et al., 2022).

Barzali et al. (2005) studied the influence of temperature and storage atmosphere of rye seeds in which seeds placed under vacuum condition at -15°C had the highest viability compared to seeds placed in normal airtight sealed packaging. In the study by Abreu et al. (2013), the packaging itself (vacuum or non-vacuum) had equal performance when stored in cold chamber condition. However, when sunflower seeds were stored in conventional condition (temperatures varying between 18°C and 20°C; and RH varying from 50% to 70%), vacuum sealed packaging was efficient in maintaining seed quality.

It was observed that in cotton seed stored in vacuum condition, had better germination, and maintained its viability more than 80% after 18 months in storage either in cold storage or room temperature while the seed in non-vacuum packaging reduced to 57% (Meena, 2017). Moisture content (MC) in non-vacuum packaging increased from 8.3% to 12% after 18 months while the seed stored in vacuum packaging maintained its MC at 8.23%. Other studies showed promising results when the seed was kept under vacuum conditions such as in groundnut (Sastry et al., 2007) and chilli (Deepa et al., 2013).

Currently, there were only two studies, one conducted by Chiu et al. (2003) where vacuum packaging was incorporated during the seed storage of sweet corn. However, in this study, sweet corn seed was only stored in partial vacuum (PV) conditions where the seeds were primed beforehand and only being stored in room temperature (25°C). The finding showed that the sweet

corn seed that had been stored in PV performed the best where it improved seed vigour and reduced free radical-related peroxidation. The activity of antioxidant enzymes such as SOD, CAT and APX was reduced after 12 months, but the rate is much slower in PV seed compared non vacuum. Apart from that, the accumulation of MDA content in vacuum-stored sweet corn seed was not as fast as nonvacuum seed, indicating slower rate of deterioration. Another study by De Camargo & De Carvalho (2008) showed that seeds stored in vacuum condition (0.1 atm) had smaller reduction in seed physiological quality than normal packaging using kraft paper for 18 months when stored in conventional storage.

Apart from the above studies, it was observed that primed rice seeds o stored in vacuum condition, had no reduction in viability after 60 days even when stored in increased storage temperature (30°C) and had equal performance with the seeds stored in low temperature (Wang et al., 2018). This showed that there is possibility of storing the seeds in normal room temperature, without compromising seed viability, as long as seeds are stored in vacuum conditions, and this, is yet to be tested in this thesis.

2.4 Seed Priming

Seed germination is very important as uniform and rapid seed emergence would provide good seedling establishment for better planting procedure. Researchers have tried many approaches and techniques to improve seed

germination and obtain better seedling establishment. Seed priming is a promising technique to fulfil the above (Dutta, 2018). It is a technique of controlling hydration and drying, that results in more rapid germination when the seeds are re-imbibed (Bradford, 1986). It allows imbibition and activation of the initial metabolic events associated with seed germination but prevents radicle emergence and growth before seeds are dried back to their original water content during seed germination to improve seed viability and vigour.

Seed priming benefited growers in many ways. Many studies had shown greater improvement in seed viability and vigour, such as maximizing seed viability, accelerated rates of emergence and germination, reducing erratic germination, improving seed vigour and seedling performance (Abdelhamid et al., 2019; Pawar & Laware, 2018; Zulfiqar, 2021).

Seed priming works around the physiology process of germination, in three crucial phases, namely Imbibition Phase (Phase 1), Lag Phase (Phase 2) and Germination Phase (Phase 3) (Paul et al., 2022). Imbibition phase is the first step where seeds will take up water rapidly to reactivate its metabolic activities in which translation and restoration of mitochondria and DNA occurs. Imbibition phase takes place at a different rate for every species, hence the need to identify the imbibition time for each species (Wierzbicka & Obidzińska, 1998). The lag phase (Phase 2) is also known as the activation and preparatory phase where the water uptake will be slowing down to almost plateau. During this phase, all the physiological and metabolic events are at

peak in which new mRNA and proteins are being synthesized, activation of all germination related enzymes and increase in respiration is observed (Paul et al., 2022). These two phases are most delicate and important to successfully prime the seeds because the process of germination is a reversible phenomenon (Ibrahim, 2019). The two phases also noted as pre-germination processes before seed enter the last phase, the germination phase (Phase 3). In normal seed germination activity, Phase 3, a germination phase in which radicle protruded from the seed coat and rapid water uptake resume rapidly to accommodate germination (Paul et al., 2022). Once protrusion of radicle takes place, it can no longer be reversed like the first two phases (Farooq et al., 2019; Marthandan et al., 2020)

Seed priming should be carried out in the first two initial stages because seeds need to be dehydrated back and rehydrating seed during the germination phase would kill the seed. Priming is carried out by soaking the seed in selected solution, for amount of time between Phase 1 and 2. Seed then will be dried back to its initial moisture content and can be stored thereafter(Paul et al., 2022) as illustrated in Figure 2.6.

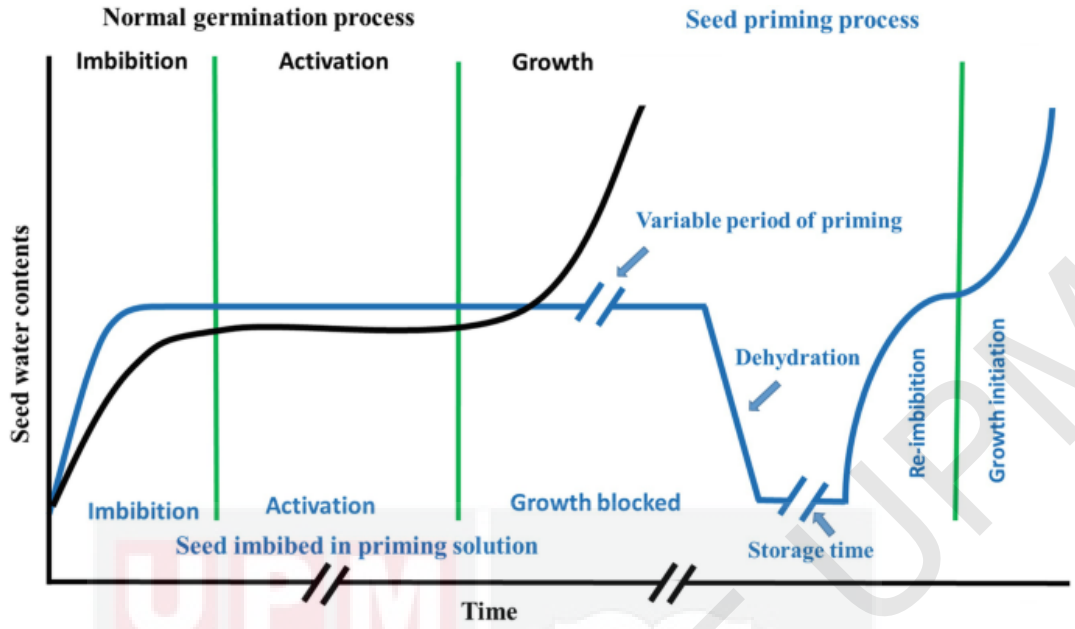

Figure 2.6: Schematic diagram of water uptake during seed germination and the seed priming process. Adapted from Ibrahim (2019).

From physiological point of view, priming helps by stimulating varieties of metabolic activities needed prior to germination, such as repair mechanism and enzymes activation. This would allow primed seeds to move to third stage (radicle protrusion) as pre-germination metabolic activities were done during the soaking time, hence benefiting the seed vigour (Ibrahim, 2019).

2.4.1 Types of Seed Priming

There are several type of seed priming methods that are commonly used: hydro-priming, halo-priming, osmo-priming, thermo-priming, chemical priming, hormo-priming, solid matrix priming, and bio-priming (Eskandari, 2013).

Hydro-priming is the simplest form of priming in which seeds are soaked in water for a period before being dried back to its initial moisture while hormo-priming involves soaking the seed in plant growth regulators or hormones such as gibberellic acid or salicylic acid. Bio-priming is also another type of priming in which plant growth promoting bacteria is used to enhance the seed performance (Sher et al., 2019).

Osmo-priming is a method of soaking seeds to a low external water potential to restrict the rate and extent of imbibition while priming that requires soaking the seeds in aerated solution of inorganic salt is called as halo-priming. Common osmo-priming and halo-priming agents include PEG, KNO_3, KCl, K_3PO_4, KH_2PO_4, $MgSO_4$, $CaCl_2$, NaCl, and mannitol among many others (Jisha et al., 2013). Solid matrix priming on the other hand involves the usage of solid medium such as vermiculite (Sher et al., 2019). The use certain natural or synthetic compound such hydrogen sulphide, melatonin, polyamines, nitric oxide, zinc oxide, H_2O_2 among many others, are classified as chemical priming (Thakur et al., 2019).

Among the factors that need to be considered are temperature, duration of treatment and the selected solution concentration (Ibrahim, 2019), therefore the selection of priming method must consider all of these factors to ensure priming can be done successfully to improve seed viability and its vigour.

2.4.2 Sweet Corn Seed Priming

Sweet corn seed has been primed with many substances ranging from the simplest form of priming (hydro-priming) to nano priming. For example, Nciizah et al. (2020) used different types of macronutrient as priming agent in which the study soaked the seeds in several compound such as zinc sulphate, sodium molybdate and boric acid at five different concentration at three different soaking time (8, 12 and 24 hours). Based on the study, the best way to prime *Zea mays* seeds is 0.01% of boric acid for 24 hours.

In Hassanzadeh et al. (2013), they used different chemical priming with 2% potassium chloride, 3% potassium nitrate, polyethylene glycol (8000) 10%, 1% potassium dehydrogenate phosphate with control) and four NaCl concentration (0, 50, 100, 150 mM) and found the best is 3% potassium nitrate for 24 hours in room temperature. Priming using potassium nitrate showed promising result when corn seed was under abiotic stress (KNO_3 for 24 hours in room temperature) (Anosheh et al., 2011) .

Hormo-priming using plant growth regulators showed that addition of plant regulators such as gibberellic acid did not improve seed quality while PEG (halopriming) increases seed viability and vigour of sweet corn when primed for 24 hours in room temperature (Pallaoro et al., 2016). The use of bio-priming using *Bacillus megaterium* also increased seed viability of sweet corn such as in study by El-Hamed et al. (2011). Priming using *Pseudomonas fluorescens* also

was found to improve seed quality of sweet corn for 24 hours at 23°C (Callan et al., 2019).

Solid matrix priming of sweet corn seed with sand (Zhao et al., 2009) and vermiculite (Chiu et al., 2003) also attempted with the best priming time at 24 hours and 36 hours respectively. Nano priming technology using zinc also gained attention with it improving seed viability and dry mass in which seeds were soaked in nano zinc for 24 hours also in room temperature (Sharifi, 2016).

Many studies above had shown that the most common soaking time and priming temperature that show a success is soaking time for 24 hours at room temperature. While all these priming agents worked, it can be expensive and difficult to obtain especially to small farmers, hence exploring cheaper option is important to make it easily accessible.

2.4.3 Hydrogen Peroxide as Osmopriming Agent

H_2O_2 is a reactive molecule that plays a dual role in plant physiological and developmental processes and in resisting stress (Wojtyla et al., 2016). H_2O_2 at proper concentration released dormancy and enhanced germination (Lariguet et al., 2013). Yet, the over-accumulation of H_2O_2 easily caused cell injury (Jeevan et al., 2015).

Antioxidant enzymes produced in plants such as SOD, CAT, ascorbate peroxidase (APX), and glutathione reductase (GR) contributed to the endogenous H_2O_2 (Hossain et al., 2015; Ślesak et al., 2007). H_2O_2 also signals directly or indirectly and is involved in many signalling pathways of gibberellic acid (GA), abscisic acid (ABA), indole-3-acetic acid (IAA) and so on (Wojtyla et al., 2016). A study by Wang et al. (2020) also demonstrate how H_2O_2 plays an important roles in signalling plant adaptation to stress response. For example, in water deficit stress, supplementation with H_2O_2 can improve wheat tolerance to drought by regulating oxidative damage response mechanism, the accumulation of osmolytes, lessening lipid peroxidation which improve plant growth and yield (Habib et al., 2020).

Priming using H_2O_2 has been done successfully before. It was found to have a positive effect on rice seed. Hemalatha et al. (2017) tried multiple concentration of H_2O_2 at 0.25%, 0.5%, 0.75% and 1:1 ratio with the priming at 0.25% showing a positive effect on seed performance especially in mitigating salt stress in rice. In drought stress, Jira-Anunkul & Pattanagul, (2020) tested different concentration of H_2O_2 at 0, 1, 2, 5, 10, and 15 mM with priming at 10 mM for 24 hours had better performance to lessen the effect of this stress on rice. Concentration at 80 mM H_2O_2 was found to be the best concentration to prime cotton seeds with soaking time at 24 hours (Santhy et al., 2014). On the other hand, priming at 10 mM of H_2O_2 for pepper seeds showed that it allows rapid germination to be in salt or drought stress, where there was a reduction of mean germination time. (MGT), improvement in seed germination rate (GR)

and germination index (GI) (Gammoudi et al., 2020). In wheat, priming with 0.1 mM of H_2O_2 is efficient in reducing the effect of salinity stress with germination viability, root and shoot dry mass as well and seedling vigour index (SVI) were improved in the study by Tania et al. (2021).

In sunflower, priming of H_2O_2 manage to alleviate the oxidative stress damage as there was an increase of antioxidant enzyme such as CAT to counter back the damage (Silva et al., 2020), even under salt stress (Silva et al., 2022). Priming with H_2O_2 also activated defence response of *Brassica juncea* seeds such as glutathione peroxidase, dehydroascorbate reductase and at the same time reducing MDA content enabling it seeds to tolerate drought-induce oxidative stress (Mohammad, 2013).

Ijaz (2012) studied the effects of H_2O_2 on grain corn and the priming with 20-40 mg/L of H_2O_2 improved emergence, vigour, and antioxidant activity and yet to be tested on sweet corn. Another study was conducted by Imran et al. (2013) also showed that combination of 2 µM of H_2O_2 with other substances such as ascorbic acid, salicylic acid and even moringa leaf extract can improve seed viability as well as seedling performance.

2.5 Summary of Literature Review

Sweet corn is famous due to its sweetness which is favourable for human consumption. This is attained by interrupting the production of several enzymes such as AGPase responsible in the conversation of sucrose to starch, resulting in higher sucrose content. However, this trait that favour human consumption is unfavourable for seed production as it can reduce the quality of sweet corn seed, especially when the seeds are stored for longer period. Among the reported problems are low germination, erratic emergence, and most importantly, low storability. Currently, seeds of sweet corn are harvested at different maturity stages depending on the varieties and it will be further dried to reduce its moisture to 8 to 10% before storage. There is a need to identify the suitable time to harvest GSH11005Y seed and the right temperature to dry, in order to maximize the seed potential. Study on this would help, not only for this particular variety but also other sweet corn seeds generally.

In Section 2.1.7, we discussed on how carbohydrate content particularly the sucrose and starch can be affected by environmental temperature. However, the focus of these studies was limited to human consumption, and this raises the question if the changes in the carbohydrate content in sweet corn kernel can be used to improve seed longevity.

Storage environment is an important aspect in maintaining seed quality. Seed deteriorations are affected by many factors, including temperature and oxygen availability. Both temperature and oxygen availability affect the rate of ageing particularly in seed damage by ROS in-toxification, peroxidation of lipid and many others. Currently many of sweet corn seed producers store seeds in airtight-sealed metalized PE bag to provide hermetic condition. However, cool temperature is still needed in long run to maintain seed storability. The idea of removing air from the packaging through vacuuming has been used in food industry and proven to improve seed storability in several crops however this has never been tested on sweet corn seed.

Nonetheless, seed deterioration itself is an irreversible process, hence, seed priming may be beneficial to reinvigorate poor seeds. Priming has been proven to help improve seed viability and vigour as well as seedling establishment. Many studies demonstrated the potential of H_2O_2 as a suitable priming agent to enhance seed germination and vigour in many crops, yet it has never been tested on aged sweet corn. This study, therefore, aims to provide a comprehensive strategy for sweet corn seed harvesting, post-harvest handling to prolong longevity and seed enhancement for better field establishment, ensuring better opportunity for seed marketing and trade.

CHAPTER 3

SEED MATURITY STAGES AND DRYING TEMPERATURE EFFECT ON SEED QUALITY OF SWEET CORN

3.1 Introduction

Presence of unique mutants in sweet corn changes the dynamics of sucrose and starch conversion during kernel development, which not only characterizes sweet corn nutritive components, but also the seed characteristics. The alteration of carbohydrate content by these mutants such as *shrunken2* (*sh*$_2$) instigates many seed quality problems as discussed in Section 2.1.5 and it hinders the availability of good quality seed of sweet corn.

It is known that various changes occur during seed development, and it influences seed viability and vigour. The time when seeds are harvested (Wang et al., 2018) plays an important role in determining its quality. Starch biosynthesis from sucrose during sweet corn kernel development had been attributed to poor quality especially in seed longevity of sweet corn seed. Hence, harvesting the cob at the right time when the sucrose is at the lowest and starch content as its optimum level may help in curbing the seed quality problem. Sucrose content usually is the highest at 18- 22 DAP (Tracy, 2000), where this is when the cob is harvested for human consumption. Although studies such in Cao et al. (2008) and Harakotr et al. (2022) stated that

germination could be observed as early as 14 DAP, but germination capability and vigour are still progressing at this point. Delayed harvesting can negatively affect seed quality (Vergara et al., 2019) while in some cases early harvest can produce good quality seeds (Wang et al., 2018). Therefore, information on the right harvest time when seed quality is at its optimum level is needed. Moisture at harvest of sweet corn cobs also affects seed quality. Gupta et al. (2005) found that harvesting at moisture between 40-50% had better germination compared to seeds harvested when moisture content was at 20-30%. Currently, sweet corn cob is harvested at different time depending on variety as reviewed in Section 2.1.6.

Post-harvest drying is needed to maintain seed quality during storage because high moisture will further cause seed deterioration (Bewley et al., 2013). From all the studies reviewed in Section 2.1.7, drying using temperature beyond 50°C will cause detrimental effects on seed viability and sweet corn seed is best dried between 30 to 45°C to avoid reduction in seed longevity (Guiscem et al., 2002; Gupta et al., 2005; Herter & Burris, 1989; Navratil & Burris, 1984).

Studies by Garwood et al. (1976), Olsen et al. (1990), Simla et al. (2010) and Bakry et al. (2015) showed an increase in seed starch content accompanied by a reduction in sucrose content when the seeds were stored between 18°C to 27°C compared to lower temperatures (1°C to 7°C). However, the dynamics of changes in carbohydrate content is unknown when sweet corn seed is subjected to drying at temperature between 30°C to 45°C and if these

temperatures influence the changes in seed sucrose and starch content during post-harvest drying.

Therefore, in this chapter, a study was conducted with the objective to identify the optimum harvest maturity stages and efficient drying temperature to improve seed quality and storability of sweet corn seeds based on the changes in sucrose and starch content.

3.2 Materials and Methods

3.2.1 Location of Study

Sweet corn variety GSH11005Y parental lines were planted at GWG Batu Arang Farm located in Batu Arang, Selangor (3.29092, 101.46328). The laboratory work and experiments were conducted at Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor (2.9836, 101.73407).

3.2.2 Plant Establishment and Seed Collection

Female line SC8501Y and the male line SC5101Y, both are *sh2s* lines, were planted at GWG Batu Arang to produce the hybrid GSG1005Y. Land was ploughed with chicken dung powder a week before sowing. Seeds were sown with two lanes of male parental line followed by four lanes of female parental line.

For plant maintenance, field was irrigated twice a day and NPK (15:15:15) fertilizer was added two weeks after sowing with a rate of five grams in the middle of two plants. In addition to NPK fertilizer, five grams of nitrogen fertilizer per plant was added three weeks after sowing, followed by potassium fertilizer twice at five grams and ten grams at seven weeks after sowing. For weed control, weeding was done manually for the first month followed by the use of herbicides (Gesaprim – A.I: Antrazine and Dual 960 – A.I: S-metaclor) until seven weeks after sowing.

Detasseling of female line was carried out between six to seven weeks after sowing to avoid self-pollination. The date of male pollen shading was recorded (pollination took place) and sweet corn cobs of female lines were harvested at five different maturity stages, 29, 32, 35, 38 and 41 DAP. During harvest, 30 fresh cobs were handpicked at each harvest where first ear of every five interval plants were brought to the Seed Laboratory at Universiti Putra Malaysia, Selangor, Malaysia within two hours after harvest for further process and testing.

3.2.3 100 Seed Weight (Fresh Seeds)

Sweet corn cobs were de-husked by removing the husk from the ear, leaving the cob with the seeds. Seeds were manually removed from the cob and 100

seeds sample from each of the maturity stages were immediately weighed. 100 seed weight is expressed in grams (g).

3.2.4 Glucose, Sucrose and Starch Content Measurement (Fresh Seeds)

Glucose, sucrose and starch content of seed harvested from different maturity stages was measured once the sweet corn seed removed from the cobs.

3.2.4.1 Determination and Assay of Glucose and Sucrose

Seed samples were ground using a pestle and mortar with liquid nitrogen to ease the grinding process. Two grams of the ground powder were homogenized with 5 mL of ethanol (95% v/v) and incubated in a water bath at 85±3°C for five minutes to inactivate endogenous enzymes. Solution volume was adjusted to 50 mL with sodium acetate buffer (50 mM, pH 4.5) before being allowed to cool down for 15 minutes with frequent vortex of 15 seconds. Next, 5 mL of this solution was transferred to a 15 mL centrifuge tube containing 2 mL of chloroform. It was then mixed vigorously using a vortex mixer for another 15 seconds before being centrifuged at 1,000 × g for 10 minutes and the supernatant was taken for determination and assay of glucose and sucrose.

For glucose assay, 0.2 mL of supernatant and 0.2 mL of sodium acetate buffer were mixed in 2 mL microcentrifuge tubes. For sucrose assay, 0.2 mL of

supernatant was mixed with 0.2 mL of invertase (8.3 U/mL) solution. The mixture was incubated at 50°C for 20 minutes. After the incubation, 3 mL GOPOD was added before a further incubation for another 20 minutes. Absorbance reading at 510 nm was taken using a micro-plate spectrophotometer (Thermo scientific MULTISKAN GO).

For every set of determination, glucose standard was prepared by mixing 0.1 mL of glucose standard (100 µg/0.1 mL), 0.3 of sodium acetate buffer (50 mM, pH 4.5) with 3 mL of GOPOD Reagent. Blank was prepared by adding 0.4 mL of sodium acetate buffer (50 mM, pH 4.5) with 3.0 mL of GOPOD Reagent). Both glucose and sucrose were calculated using (Kumar et al., 2010) and expressed in g /100 g seeds. Glucose content was calculated using the following formula;

$$Glucose\ (g/100\ g)\ =\ \Delta A\ \times\ F\ \times\ 250\ \times\ 200\ \times\ \frac{1}{1000}$$

Sucrose content was calculated using the following formula:

$$Sucrose\ (g/100\ g)\ =(\Delta B\ -\ \Delta A)\ \times\ F\ \times\ 250\ \times\ 200\ \times\ \frac{1}{1000}$$

Where;

ΔA = Absorbance for 0.1 mL of sample + acetate buffer

ΔB = Absorbance for 0.1 mL of sample + invertase

F = 0.056 (µmoles of glucose) / Absorbance of glucose control

3.2.4.2 Determination and Assay of Starch

For starch assay, 0.1 g of seed sample is added into 0.2 mL of aqueous ethanol (80% v/v) and mixed thoroughly using a vortex mixer in a 15 mL centrifuge tube. Next, 2 mL of cold sodium hydroxide (1.7 M) solution was added and placed in an ice-water bath for 30 minutes with frequent mixing using a vortex. Afterward, 8 mL of sodium acetate buffer (600 mM, pH 3.8) was added. Immediately 0.1 mL of undiluted thermostable α-amylase and 0.1 mL of undiluted amyloglucosidase (AMG) were added to the solution before incubation at 50°C for 30 minutes and centrifuged at 13,000 rpm for five minutes. After that, 0.1 mL of the supernatant was mixed with 3 mL of glucose determination reagent (GOPOD) before further incubation for 20 minutes at 50°C. Absorbance reading at 510 nm was taken using micro-plate spectrophotometer (Thermo scientific MULTISKAN GO). For every set of determination, glucose standard was prepared (0.1 mL of glucose standard (1.0 mg/mL) with 3 mL of GOPOD Reagent) while blank is prepared by adding 0.1 mL of acetate buffer (100 mM, pH 5.0) with 3.0 mL of GOPOD Reagent). Starch content was calculated using formula by (McCleary & Monaghan, 2002) as below and expressed in g /100 g seeds.

$$Total\ starch\ (g/100\ g) = \Delta A \times F \times 102 \times D \times \frac{1}{1{,}000} \times \frac{100}{W} \times \frac{162}{180}$$

Where;

ΔA = absorbance of sample against reagent blank

F = 0.056 (µmoles of glucose) / Absorbance of glucose control

102 = volume correction (0.1 mL taken from diluted sample volume (DSV), (10.2 mL sample solution)

D = further dilution (when needed if the absorbance reading below 0.1)

3.2.5 Seed Drying

Sweet corn cobs of different maturity stages were then placed into hot air oven with three different temperatures, 35°C, 40°C and 45°C to dry the seeds. Time taken to reduce the seed moisture to 8 - 10% was observed. Seeds were then shelled from the cobs and were kept in airtight-sealed bag at 4°C throughout the study.

3.2.6 Determination of Moisture Content

Moisture content was determined for every maturity stages before drying and every four hours during seed drying until it reaches 8 - 10%. Determination of moisture content was conducted using ISTA (2017) and Chiu et al. (2002). Initially an aluminium petri dish was weighed and recorded as W1. 25 seeds were coarsely ground and placed into the aluminium petri dish and the weight was recorded as W2. Seeds were dried using hot air oven at 130±3°C for 4 hours. Upon drying, it was immediately placed into a desiccator

for 10 minutes before the aluminium petri dish was being weighed again, W3. The moisture content was calculated using the following formula and expressed in percentage of dry weight basis (%).

$$Moisture\ content\ (\%) = \frac{W2 - W3}{W2 - W1} \times 100$$

3.2.7 100 Seed Weight (Post Drying)

Sample containing 100 dried seeds from each of the maturity stages that had been shelled earlier were weighed to get 100 seed weight. 100 seed weight of dried seeds is expressed in grams (g).

3.2.8 Seed Germination Test and Measurement of Seed Vigour

Seed germination test and measurement of seed vigour was conducted once post-harvest drying was done and it was repeated every three months during storage. Germination test was conducted using sand as substrate (ISTA, 2017). The sand was washed and sterilized using the hot air oven at a temperature of 150°C for five hours. The germination box (13.5 cm x 13.5 cm x 6 cm) was surface sterilized using ethyl alcohol (75% v/v) and then 800 g of the sterilized sand was placed into each germination box. The sand was moistened with 100 mL of distilled water and mixed thoroughly. Seeds (25 per box/replicate)

were sown at 2 cm depth. Germinated seeds were counted daily for seven days, and the data was used to calculate the following.

3.2.8.1 Final Germination Percentage

Final germination percentage (FGP) was calculated based on the final germination observed at seventh day using the following formula (ISTA, 2017):

$$\text{FGP (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seed sown}} \times 100$$

3.2.8.2 Mean Germination Time

MGT is an index for germination rate, and it is used to indicate germination uniformity and vigour of seeds. The lower the MGT, the better the quality of the seeds in terms of time taken for the seed to germinate on a given day. MGT was calculated using the following formula (Kader, 2005):

$$\text{MGT (day)} = \frac{\Sigma\,(n \times d)}{\Sigma\,N}$$

where n = number of seeds germinated on each day, d = number of days from the beginning of the test, and N = total number of seeds germinated at the termination of the experiment.

3.2.8.3 Germination Rate Index

Germination Rate Index (GRI) is calculated as follows (Kader, 2005):

$$GRI = G1/1 + G2/2 + \cdot\cdot + Gx/x$$

Where G1 = Germination percentage × 100 at the first day after sowing, G2 = Germination percentage × 100 at the second day after sowing.

3.2.8.4 Germination Index

GI is one of the index to measure seed vigour. The higher the GI, the better it is. GI can be calculated using the following formula (Kader, 2005):

$$GI = (7 \times n1) + (6 \times n2) \ldots . + (1 \times n7)$$

Where *n*1, *n*2 ... *n*7 is the number of seeds germinated on the first, second and subsequent days until the seventh day.

3.2.8.5 Coefficient Velocity of Germination

Coefficient velocity of germination (CVG) is a measurement of germination rate by considering the germination percentage and the time taken to seed

germination. CVG was calculated using the following formula (Scott et al., 1984):

$$\text{CVG} = \frac{(G_1+G_2+\cdots+G_n)}{(1\times G_1+2\times G_2+\cdots+n\times G_n)} \text{ X } 100$$

Where G is the number of germinated seeds and n is the last day of germination

3.2.8.6 Seedling Vigour Index

SVI can be used to evaluate seedling vigour. SVI was calculated using the following mathematical expression based on Abdul-Baki and Anderson (1970);

$$\text{SVI} = \text{seedling length (cm)} \times \text{percentage of germination}$$

3.2.8.7 Measurement of Seedling Length

All germinated seedlings on seventh day after sowing were arranged based on the length and the five median seedlings per replicate was selected for seedling performance analysis. Seedling length (shoot and root) was measured using a ruler and expressed in centimetre (cm). Root length was measured from the tip of the longest root to the base of hypocotyl while shoot length was

measured by measuring the shoot from the end of the longest primary leaf to the base of the hypocotyl.

3.2.8.8 Measurement of Shoot and Root Dry Weight

Sample used for seedling length measurement were separated into shoot and root and dried at 65°C for 48 hours to get the root and shoot dry weight (Brar et al., 2019), expressed in grams (g).

3.2.9 Electrical Conductivity of Seed Leachate

25 seeds for each treatment were weighed, following which it was placed in 75 mL of deionized water in a beaker and left for 24 hours at 20°C. After 24 hours, the seed leachate was measured using a digitalized conductivity meter (labCHEM-CP conductivity meter) and expressed in µS /cm /g (Thant et al., 2017).

3.2.10 Glucose, Sucrose and Starch Content Measurement (Dried Seeds)

Glucose, sucrose and starch content measurement of dried seed were conducted based on protocol describe in Section 3.2.4.13.2.4.2.

3.2.11 Determination and Assay of Antioxidant Enzymes

Assay for antioxidant enzymes was prepared using the method of Abuelsoud et al. (2020). First, MES-KOH (50 mM, pH 6.0) extraction buffer was prepared by mixing 2.665 g of MES to 250 mL of distilled water and pH was adjusted to 6.0 using KOH solution. Then, 100 mL of 50 mM MES-KOH (pH 6.0) buffer were taken and added with 40 mM of KCl (0.298 g) and 2 mM of $CaCl_2$ (0.022 g).

Next, seeds were ground in a fine powder using liquid nitrogen with a pestle and mortar. A sample of 0.15 g was mixed with 1.5 mL of ice-cold MES-KOH (50 mM, pH 6.0) extraction buffer in 2 mL microcentrifuge tube followed by centrifuging at 16,000 × *g* in 4°C for 20 minutes. Sample of supernatant was taken for the antioxidant enzymatic assay.

3.2.11.1 Measurement of Catalase (EC 1.11.1.6)

CAT activity was measured by decomposition of H_2O_2 by using the method of Aebi (1984). For CAT assay, the reaction mixture consisted of 50 mL potassium phosphate buffer (50 mM, pH 7.0) and 128µL of 30% H_2O_2 (25 mM). To prepare potassium phosphate buffer (50 mM, pH 7.0), 0.421 g of KH_2PO_4 and 0.332 g of K_2HPO_4 were mixed into 100 mL of distilled water. pH was adjusted to 7.0 using KOH or HCl. Then, 180 µL of the said reaction mixture was added to 20 µL of sample supernatant. Absorbance reading at 240 nm

using micro-plate spectrophotometer (Thermo scientific MULTISKAN GO) was recorded.

CAT activity was calculated with the following equation using the extinction coefficient of 43.6 m /cm. The CAT activity unit was expressed as per milligram of extractable fresh weight (µmol/min/mg/FW).

$$CAT\ (\mu mol/min/mL) = \frac{(\Delta 240/min)\ \times total\ volume\ \times 100}{43.6\ \times enzyme\ volume}$$

$$CAT(\mu mol/min/mgFW) = \frac{\mu mol/min/mL}{mg/mL\ enzyme}$$

3.2.11.2 Measurement of Guaiacol Peroxidase (EC.1.11.1.7)

For POD assay, protocol by Maehly & Chance, (1954) was used in which a reaction mixture consisted of 50 mL potassium phosphate buffer (50 mM, pH 6.0), 120 µL of 30% H_2O_2 (25 mM) and 149 µL of guaiacol was prepared. Next, 2 µL of sample supernatant was added to 198 µL of this reaction mixture and absorbance reading was taken at 470 nm using the spectrophotometer before.

The activity of the POD enzyme was measured in milligrams of extractable fresh tissue using the following equation (nmol/min/mg FW).

$$POD\ (nmol/min/mL) = \frac{(\Delta 470/min)\ \times total\ volume\ \times 100}{26.6\ \times enzyme\ volume}$$

$$POD(nmol/min/mg\ FW) = \frac{nmol/min/mL}{mg/mL\ enzyme}$$

3.2.12 Determination of Malondialdehyde

For lipid peroxidation, MDA was measured using Stewart & Bewley (1980). First, two grams of seed sample was homogenized using 2 mL of distilled water before being centrifuged at 10,000 × g for 15 minutes to get supernatant. Second, 3 mL of reaction mixture of 1 mL of supernatant, 2 mL of TBA/TCA (0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid) solution. The mixture was incubated for 30 minutes in water bath with temperature of 95±3°C. After that, it was placed in ice tray immediately after the incubation to stop the reaction before reading was taken at 450, 532 and 600 nm using micro-plate spectrophotometer (Thermo scientific MULTISKAN GO) and MDA was estimated and expressed in μM/g of FW using the following formula.

$$MDA\ (\mu M/g\ /\ FW\ ) = 6.45\ \times\ (\Delta 532 - \Delta 600) - 0.56\ \times\ \Delta 450$$

where,

Δ532 = Absorbance reading at 532 nm

Δ600 = Absorbance reading at 600 nm

Δ450 = Absorbance reading at 450 nm

3.2.13 Experimental Design and Data Analysis

The experiments were conducted in a factorial complete randomized design (CRD) with four replications. The two factors studied were the harvesting time and post-harvest drying temperature. The seed was then stored for a year and the quality was measured quarterly to measure seed storability performance. Data were subjected to Analysis of Variance (ANOVA) with post-hoc comparison were conducted using Tukey Test. All statistical analysis were conducted using SAS Version 9.4 (SAS Institute Inc., 2012).

3.3 Results

3.3.1 Pre-Storage Characteristics and Performance

Seed characteristics upon harvest, during drying and post-harvest drying of GSH11005Y sweet corn were as follows. Results of initial performance based on seed quality, seedling performance and biochemical activities are also shown in this section.

3.3.1.1 100 Seed Weight (Fresh Seed)

Hundred seed weight of fresh seed is illustrated in Figure 3.1. At 29 DAP, fresh seed weight was at 28.43 g before reaching maximum fresh weight at 32

DAP (29.5 g). Seed fresh weight eventually dropped gradually with minimum observed at 26.77 g at 41 DAP (ANOVA is referred in Appendix 3.1).

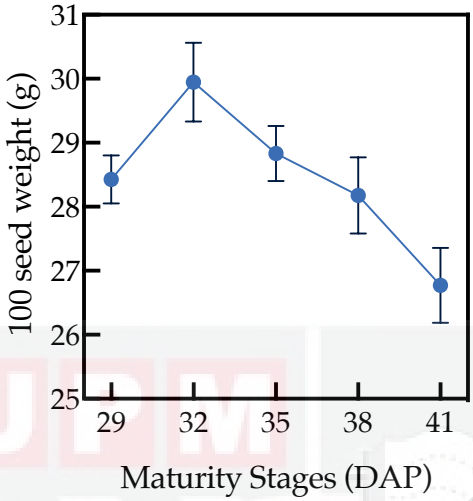

Figure 3.1: Weight of fresh hundred sweet corn seed harvested between 29 to 41 DAP. Overlapped bars are not significantly different at P > 0.05 using Tukey Test.

3.3.1.2 Changes of Glucose, Sucrose and Starch Content (Fresh Seed)

Glucose content post-harvest and before drying showed a steadily declining trend. The highest glucose content was at 29 DAP which is around 2.5 g /100 g of seed, a very small fraction compared to sucrose and starch content. The seed glucose content went down gradually with the lowest amount observed at 41 DAP which was only at 0.42 g /100 g of seed. Changes in glucose content based on the maturity stages of sweet corn seed is illustrated in Figure 3.2 (ANOVA is referred in Appendix 3.2).

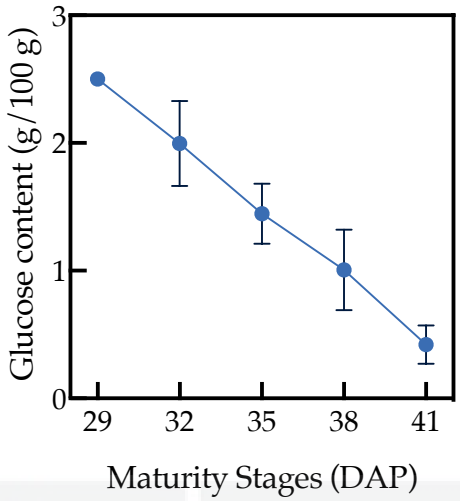

Figure 3.2: Glucose content of sweet corn seeds harvested at five different maturity stages. Overlapped bars are not significantly different at P > 0.05 using Tukey Test.

Sucrose content in Figure 3.3 (ANOVA is referred in Appendix 3.3) also had a downward trends throughout 29 to 41 DAP. In this study, the maximum amount of sucrose was observed at 29 DAP (11.12 g /100 g) followed by 32 and 35 DAP at 10.26 g /100g and 7.6 g/ 100g. Low seed sucrose was observed between 38 to 41 DAP with the lowest observed at 38 DAP at 5.38 g /100 g of seeds.

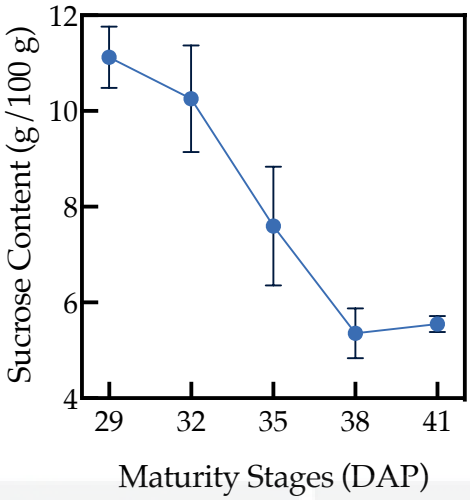

Figure 3.3: Sucrose content of sweet corn seeds harvested at five different maturity stages. Overlapped bars are not significantly different at P > 0.05 using Tukey Test.

Starch that is transformed from soluble sugar (sucrose) in the sweet corn seed had increment from 29 (13.73 g /100 g) to 32 DAP (15.49 g /100 g) where it peaked before it reduced gradually until 41 DAP (13.73 g /100 g). Starch changes in sweet corn seed is illustrated in Figure 3.4 (ANOVA is referred in Appendix 3.4). These finding will be compared with the glucose, sucrose, and starch content after drying to understand how the changes in these three carbohydrates compound affect the seed quality and seed storability.

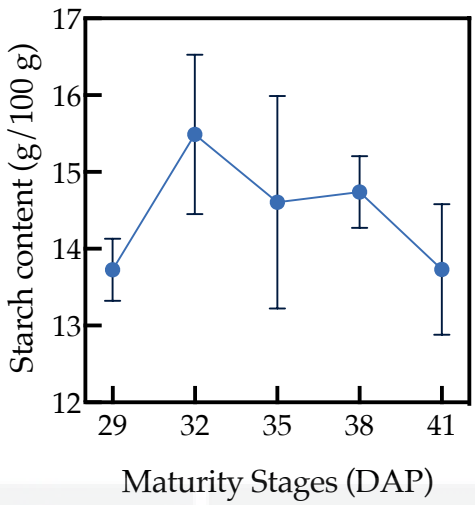

Figure 3.4: Starch content of sweet corn seeds harvested at five different maturity stages. Overlapped bars are not significantly different at P > 0.05 using Tukey Test.

3.3.1.3 Seed Moisture Content at Harvest

Based on the ANOVA in Appendix 3.5, seed moisture content was the highest at 29 DAP at 60% and it reduced to 40% at 41 DAP and the seed dry weight was the highest at 29 DAP before it reduced and maintained as the maturity goes, this can be seen in Figure 3.5. Maximum seed dry weight before post-harvest drying was at 9.56 g /100 seeds before reduced and slightly stagnant between 35 to 38 DAP (7.07 - 7.22 g /100 seeds). This indicate that the seed filling was already complete by the time the seeds were harvested at 29 DAP or before that.

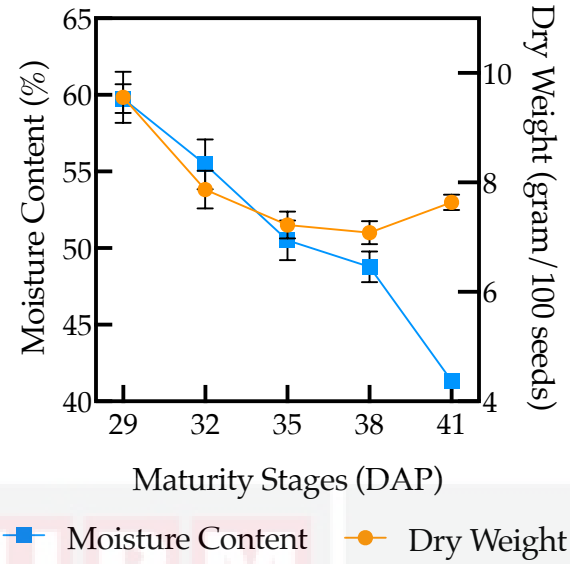

Figure 3.5: Initial moisture content and dry weight of seeds harvested at different maturity stages. Overlapped bars are not significantly different at P > 0.05 using Tukey Test.

3.3.1.4 Changes in Moisture Content upon Drying

Regardless of temperature or maturity stage, drying consistently reduced the seed moisture content, with the time taken to reach the desired 8% moisture level varying according to maturity stages and drying temperatures (Figure 3.6 and Appendix 3.6).At 35°C, seeds required the longest drying time to reach 8% moisture content across all maturity stages. For example, seeds harvested at 29 DAP took 144 hours to dry to the target moisture, while seeds harvested at 41 DAP took 102 hours. The early-harvested seeds (29 DAP) required more time due to their higher initial moisture content of 59.8%, compared to the later stages.

In contrast, seeds dried at 40°C and 45°C exhibited almost identical drying rates. Seeds harvested at 29 DAP and dried at these temperatures required only 96 hours to achieve 8% moisture content, which was a 33.3% reduction in drying time compared to those dried at 35°C. The fastest drying time was observed in seeds harvested later, particularly at 38 DAP and 41 DAP, where the drying time dropped to just 64 hours when dried at 40°C or 45°C. This rapid drying was possible due to the lower initial moisture content in these seeds (around 41.34% for seeds harvested at 41 DAP), which contributed to a 42.1% faster drying time compared to the early-harvested seeds.

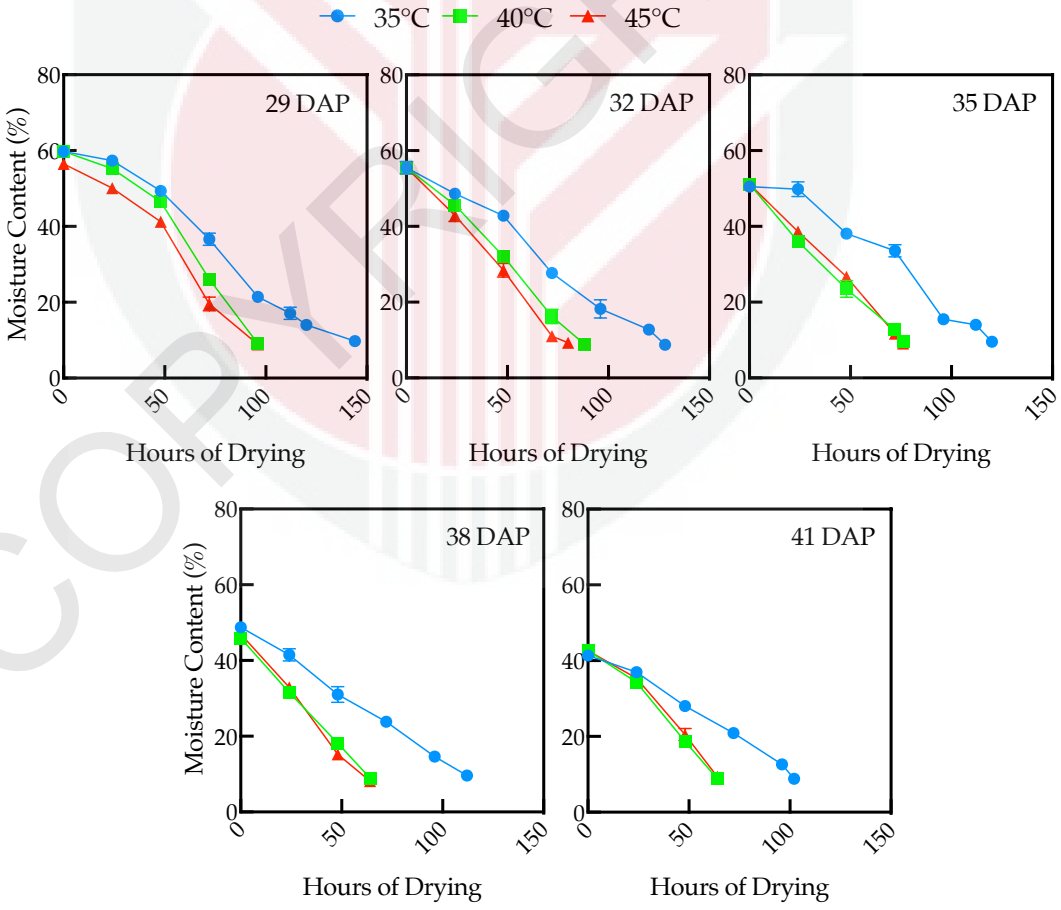

Figure 3.6: Changes in seed moisture content upon drying using three different temperatures on corn cobs harvested at different maturity stages. Overlapped bars are not significantly different at P > 0.05 using Tukey Test.

3.3.1.5 100 Seed Weight (Post Drying)

Seed weight of sweet corn after drying is shown in Figure 3.7 (ANOVA is referred in Appendix 3.7). Drying at 35°C had the highest 100 seed dry weight, followed by 40°C and the lowest was observed when the seed dried at 45°C. Seeds collected 32 DAP followed by 35 DAP and 29 DAP had the highest seed weight while for seeds harvested at 41 DAP. Maximum seed dry weight was observed at 32 DAP when dried at 35°C (10.84 g) followed by seeds at 35 DAP, (10.1 g) which also dried at 35°C. Maximum dry weight of sweet corn dried at 40°C was observed when seeds harvested at 32 DAP (10.05 g). The lowest seed dry weight was obtained from seeds harvested at 41 DAP with all drying temperature had no difference in influencing the seed weight.

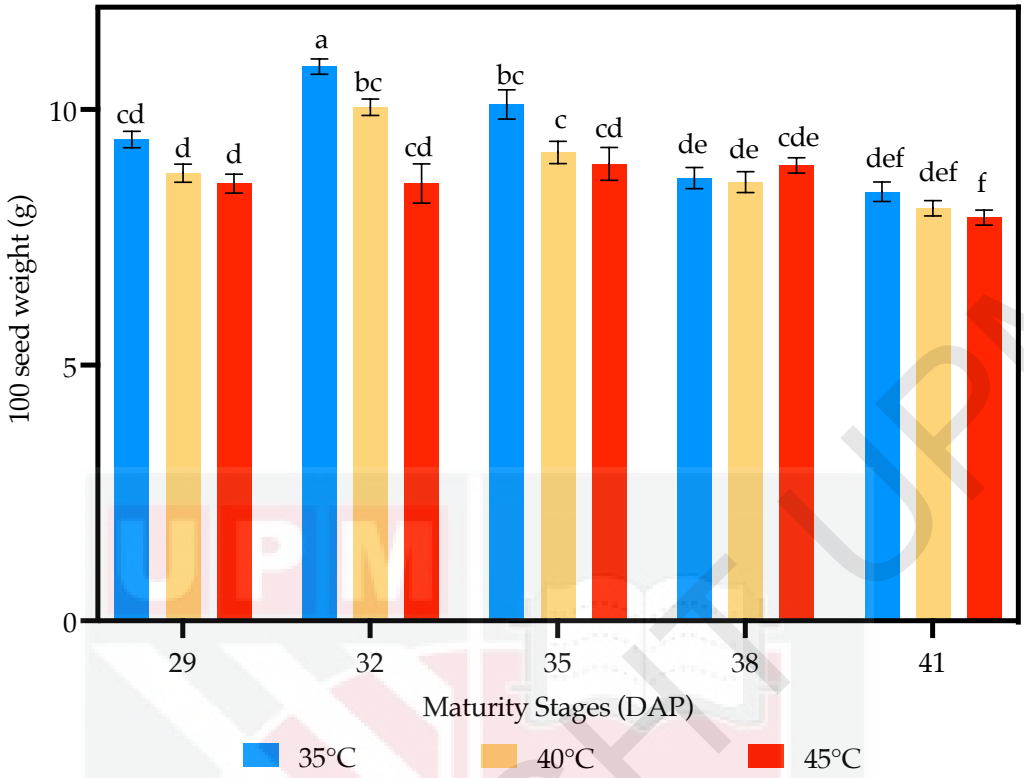

Figure 3.7: Seed weight of different maturity stages of sweet corn GSH11005Y after drying in three temperature regime. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

3.3.1.6 Final Germination Percentage

Based on the ANOVA in Appendix 3.8, both factors, maturity stages and drying temperature did not significantly affect seed viability. The FGP for all treatments was high recording more than 97%. There was no interaction between maturity stages and drying temperature on seed quality in terms of vigour and seedling quality. The summary of seed viability is shown in Table 3.1.

Table 3.1: Summary of seed viability and vigour of sweet corn collected at different maturity stages and dried at different drying temperature.

Source of Variation	Seed Viability and Vigour				
	FGP	**MGT**	**GI**	**GRI**	**CVG**
	(%)	days	-	% / day	-
Maturity Stages	ns	**	**	**	**
29 DAP	99.33	3.71 b	426.00 a	27.44 a	27.01 a
32 DAP	99.00	3.72 b	423.00 a	27.21 a	26.89 a
35 DAP	98.33	3.73 b	419.67 a	26.84 ab	26.86 a
38 DAP	99.33	3.96 a	401.00 b	25.58 bc	25.34 b
41 DAP	100	4.00 a	400.00 b	25.31 c	25.03 b
Drying Temperature	ns	ns	ns	ns	ns
35°C	99.00	3.86	411.00	26.20	25.95
40°C	99.00	3.79	417.80	26.73	26.44
45°C	97.80	3.82	413.00	26.50	26.30
Maturity Stages × Drying Temperature	ns	ns	ns	ns	ns

FGP is final germination percentage, **MGT** is mean germination time, **GI** is germination index, **GRI** is germination rate index and **CVG** is coefficient velocity of germination.
** indicates highly significant differences at $P \leq 0.0001$.
ns indicates no significant differences.
means with same letter vertically in each of seed quality parameters and source of variation sections are not significantly different at $P > 0.05$ using Tukey Test.

3.3.1.7 Mean Germination Time

Based on the summary of ANOVA in Table 3.1 and Appendix 3.9, there was no significant interaction observed between the two factors (maturity stage and drying temperature) on seed MGT. However, maturity stages significantly affected seed MGT, while drying temperature had no significant impact. As shown in Table 3.1, the shortest MGT was observed in seeds harvested at 29 DAP, with a germination time of only 3.71 days. This was followed by seeds harvested at 32 DAP (3.72 days) and 35 DAP (3.73 days), with no significant differences between these three stages. In contrast, seeds harvested later, at 38 DAP and 41 DAP, took significantly longer to germinate, with MGTs of 3.96 days and 4.00 days, respectively. This represents a 6.7% increase in MGT for seeds harvested at 38 DAP compared to 29 DAP, and a 7.8% increase for seeds harvested at 41 DAP.

3.3.1.8 Germination Rate Index

Same trend in GRI was observed where no interaction was observed in this parameter. However, there was a significant effect of maturity stages on GRI while the temperature of which seeds were dried has no effect. Details of each treatment effect on GRI is summarized in Table 3.1 and Appendix 3.10. Seeds harvested between 29, 32 and 35 DAP had the same performance, 27.4%, 27.2% and 26.8% per day while seeds harvested later had a lower number of seeds germinating in a day which only at 25.6%.

3.3.1.9 Germination Index

In Table 3.1 and Appendix 3.11, there was no interaction observed between the two factors studied. Maturity stages showed a significant effect on the seed GI. Likewise, all drying temperature did not show any significant difference among them. There was no significant difference between the seeds harvested between 29 DAP to 35 DAP based on their GI with the best GI was observed at 29 DAP (426 points) while the lowest when the seed harvested late as such in 41 DAP with 400 points.

3.3.1.10 Coefficient Velocity of Germination

There was no significant interaction or effect of drying temperature on CVG, as detailed in Table 3.1 and Appendix 3.12. However, maturity stages had a significant effect on seed's CVG. Seeds harvested between 29 to 35 DAP exhibited more rapid germination compared to those harvested between 38 to 41 DAP. The best performance was observed in seeds harvested at 29 DAP with a CVG of 27.01, followed closely by seeds harvested at 32 DAP (26.89) and 35 DAP (26.86), with no significant differences between them. The lowest CVG was observed in seeds harvested at 41 DAP (25.03), which showed a 7.3% decrease in germination speed compared to seeds harvested at 29 DAP. Seeds harvested at 38 DAP (25.27) also showed a similar 6.7% decrease in germination speed compared to those harvested at 29 DAP.

3.3.1.11 Seedling Vigour Index

Based on ANOVA in Appendix 3.13, there was no interaction between the two factors, but SVI was affected significantly by seed drying temperature. Figure 3.8 shows the performance of seeds dried at different temperature based on SVI. The seeds dried at 40°C has the best performance at 2,650.85. Although seeds dried at 35°C (2,623.04) was not significantly different with 40°C, but it was also not significantly different with seeds dried at 45°C (2,553.22). Maturity stages on the other hand had no significant effect on SVI.

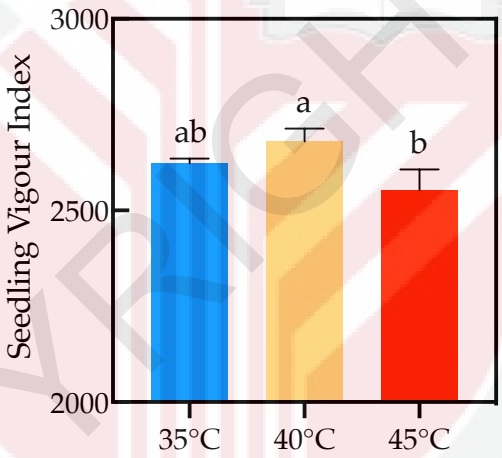

Figure 3.8: Seedling vigour index based on drying temperature of sweet corn seed. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

3.3.1.12 Seedling Length

There was no interaction observed but drying temperature influenced the seedling length of sweet corn (Refer Appendix 3.14). Trend that was observed in SVI before also recurring in the seedling length where the seeds dried at 40°C (26.97 cm) had the longer seedling compared to 45°C which performed

the lowest (25.84 cm) as shown in Figure 3.9. Seedling length also was not affected by the seed maturity stages.

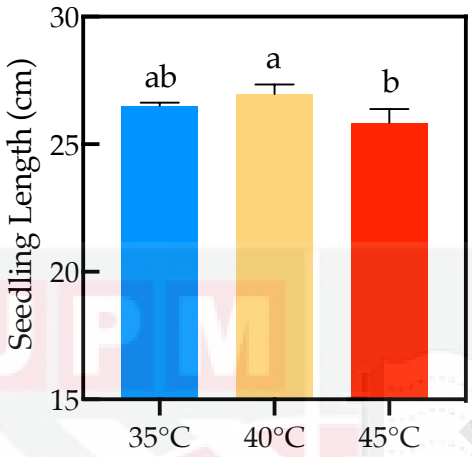

Figure 3.9: Seedling length of sweet corn dried at three different drying temperature. means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

3.3.1.13 Seedling Root Dry Weight

Based on ANOVA in Appendix 3.15, there was a significant effect of drying temperature on seedling root dry weight. However, there was no significant effect of seed maturity stages on seedling root dry weight with no interaction was observed between the two. Based on root dry weight, seeds dried at 40°C (0.0374 g /seedling) provides the highest dry weight followed by 35°C (0.035 g /seedling) and the lowest was observed when the seeds dried at 45°C (0.0311 g /seedling) as in Figure 3.10.

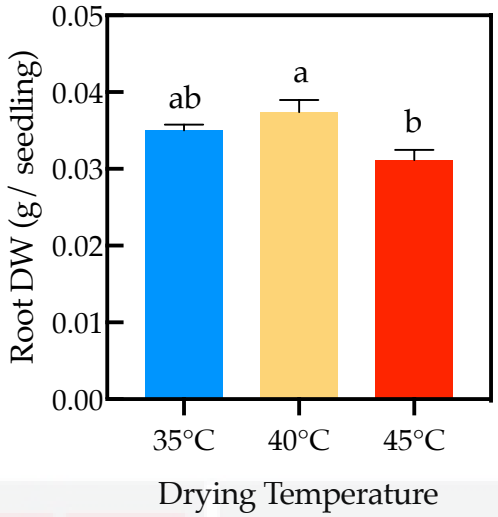

Figure 3.10: Seedling root dry weight as measured based on the different drying temperature. means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

3.3.1.14 Seedling Shoot Dry Weight

There was no significant interaction was observed between the two factors on seedling shoot dry weight (Refer Appendix 3.16). This parameter had same trend as in seedling root dry weight in which that only seed drying temperature influenced shoot dry weight while the seed maturity stages had no effect. From Figure 3.11 below, the seeds dried between 35°C (0.0254 g /seedling) and 40°C (0.0255 g /seedling) performed better compared to the seeds dried at 45°C (0.0236 g /seedling).

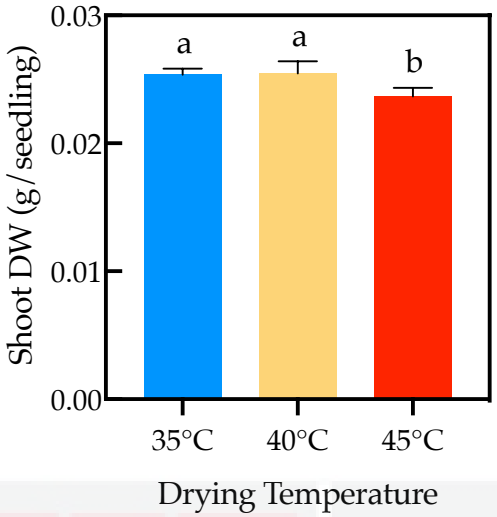

Figure 3.11: Seedling shoot dry weight as measured based on three different drying temperature. means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

3.3.1.15 Electrical Conductivity of Seed Leachate

Based on ANOVA in Appendix 3.17, both factors had no significant interaction on the amount of electrical conductivity (EC) seed leachate measured. Likewise, maturity stages and drying temperature independently affected the number of leachates from the seed membrane from Figure 3.12a, the highest amount of seed leachate was observed when the seeds were harvested early, 29 DAP (47.94 µS /cm /g). Seeds harvested between 32 and 35 DAP were not significantly different (35.58 - 35.69 µS /cm /g). The lowest amount of seed leachate was observed when the seeds were harvested late between 38 and 41 DAP with the lowest amount being observed at 41 DAP at 25.61 µS /cm /g. Meanwhile, seeds subjected to 45°C had the highest amount of seed leachate, indicated by the poor performance of the seed membrane integrity (39.54 µS /cm /g). Drying temperature influenced the membrane integrity with seeds

being dried at 40°C having values of 33.89 µS /cm /g while the seeds dried at 35°C had the lowest amount of seed leachate at 29.88 µS /cm /g. This is illustrated in Figure 3.12b.

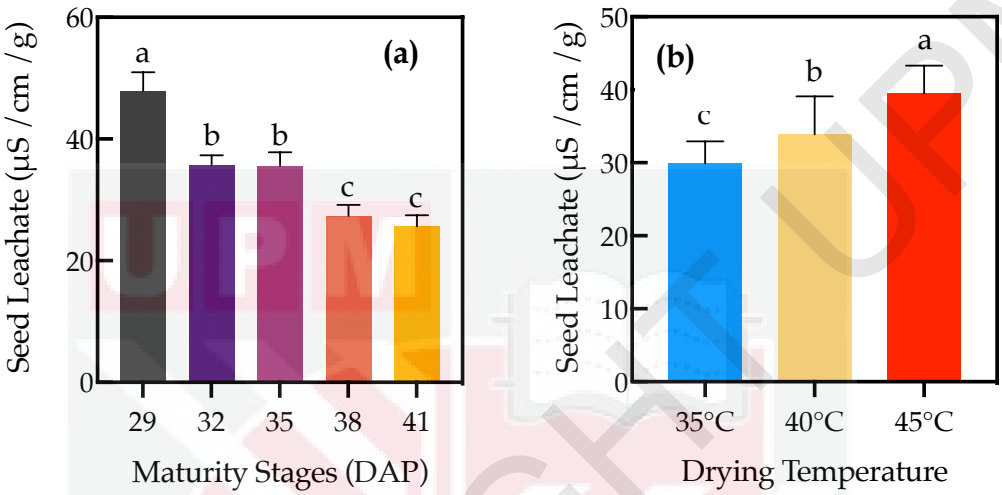

Figure 3.12: Electrical conductivity of seed leachate based on maturity stages (a) and drying temperature (b). Means with the same letter is not significantly different. means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

3.3.1.16 Changes in Glucose, Sucrose and Starch

As can be seen in Figure 3.13, , there was a significant interaction between maturity stages and drying temperature on glucose content (Refer Appendix 3.18). In seeds harvested early, glucose content was higher and decreased as harvest time was delayed. For example, glucose content in seeds harvested at 29 DAP was 2.5 g/100 g of seed weight, which represented a 70% reduction to 0.75 g/100 g in seeds harvested at 41 DAP.

After drying, glucose content further decreased across all maturity stages. For seeds harvested at 29 DAP, drying temperature significantly affected the glucose content. Seeds dried at 40°C showed a reduction of 56.4%, dropping from 2.5 g/100 g to 1.09 g/100 g, while seeds dried at 45°C experienced a 45.2% decrease, from 2.5 g/100 g to 1.37 g/100 g. In contrast, seeds dried at 35°C showed a more substantial reduction of 76%, dropping from 2.5 g/100 g to 0.59 g/100 g. For seeds harvested at 32 DAP, no significant differences were observed between drying temperatures. Seeds dried at 35°C (0.55 g/100 g), 40°C (0.52 g/100 g), and 45°C (1.08 g/100 g) showed similar glucose content, with reductions of 78% to 80% from the initial glucose content. For seeds harvested at 35, 38, and 41 DAP, glucose content decreased after drying, but drying temperature had no significant effect on the reduction. The decrease in glucose content was approximately 70-80% for all drying temperatures.

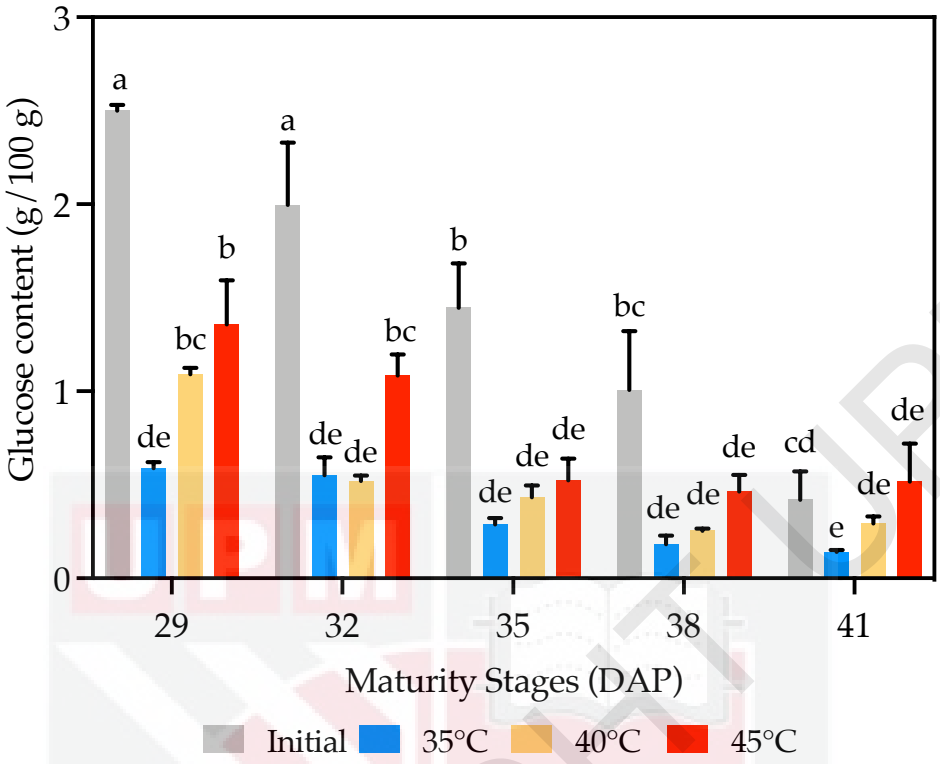

Figure 3.13: Changes in glucose content before and after drying. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

In terms of sucrose content, there was a significant interaction between harvest time and drying temperature. Sucrose content significantly decreased from 29 DAP (11.12 g/100 g) to 41 DAP (5.55 g/100 g) before drying, representing a 50% reduction in sucrose content ((Refer Figure 3.14 and Appendix 3.19).

After drying, in contrast to glucose, sucrose content increased significantly for all maturity stages. Drying temperature had a notable effect on seeds harvested at 29 DAP, where seeds dried at 45°C accumulated the highest sucrose content, showing an increase of 13.3% (from 11.12 g/100 g to 12.60 g/100 g) compared with seeds dried at 35°C (11.78 g/100 g).

For seeds harvested between 32 DAP and 41 DAP, there were no significant differences in final sucrose content across the different drying temperatures once the desired moisture content was reached (Refer Figure 3.14 and Appendix 3.20).For 29 DAP seeds, final sucrose content was above 10.76 g/100 g for all drying treatments, reflecting a decrease between 3.2% to 3.8% from the initial sucrose content. In contrast, at 41 DAP, the final sucrose content was between 9.00 g/100 g and 9.8 g/100 g, showing a relative increase of 62% to 76% from the initial sucrose content (5.55 g/100 g). Despite the low initial sucrose content in seeds harvested at 41 DAP, drying treatments resulted in a noticeable increase in sucrose

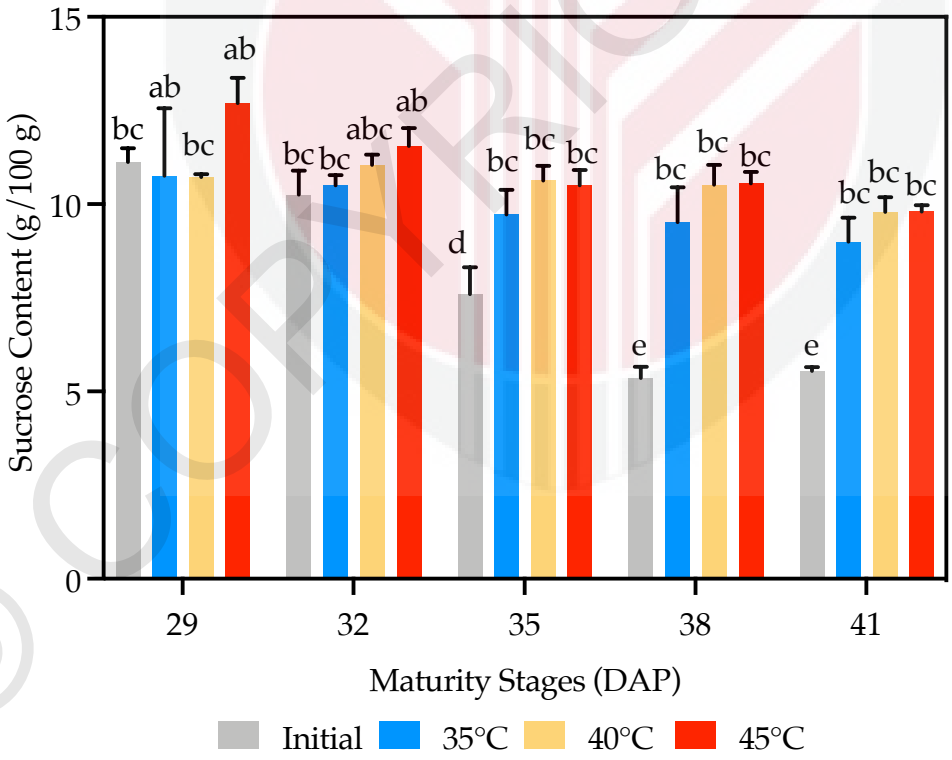

Figure 3.14: Changes in sucrose content before and after drying. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

Based on starch content in Figure 3.15 and Appendix 3.20, there was an interaction between maturity stages and drying temperature. There was no significant difference on starch content based on maturity stages before drying ranging from 13.73 to 15.49 g /100 g between 29 DAP 41 to DAP. However, there was a significant reduction in starch content for all maturity stages after drying. Drying at 45°C caused the highest reduction in starch content for all maturity stages compared to 35°C and 40°C except at 29 DAP where no significant difference was observed. The smallest reduction was observed in seeds harvested at 32 DAP at 40°C with only 0.87 g /100 g reduction. The highest reduction was observed in 38 DAP where starch content drops from 14.74 g to 6.31 g /100 g of seed.

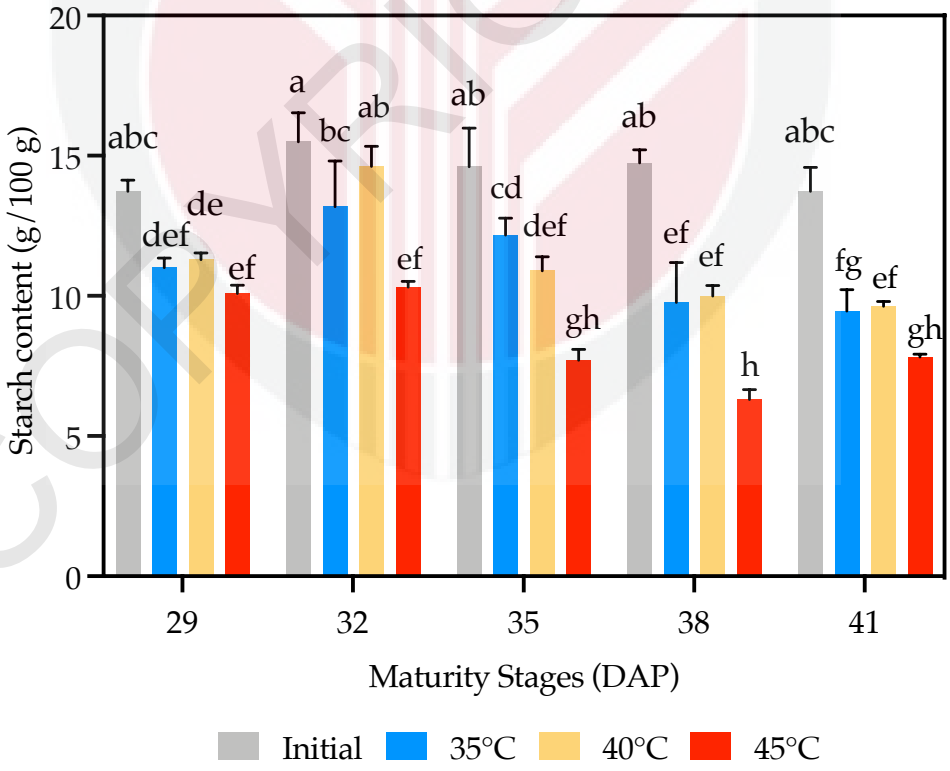

Figure 3.15: Changes starch content before and after drying. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

3.3.1.17 Antioxidant Enzymes Activities

In CAT activity (Figure 3.16), no interaction was observed with only maturity stages showed a significant effect (Refer Appendix 3.21). Highest amount of CAT was observed when the seeds were harvested at 32 DAP (7.23 µmol /min /mg FW) while the lowest was observed at 41 DAP (5.57 µmol /min /mg FW).

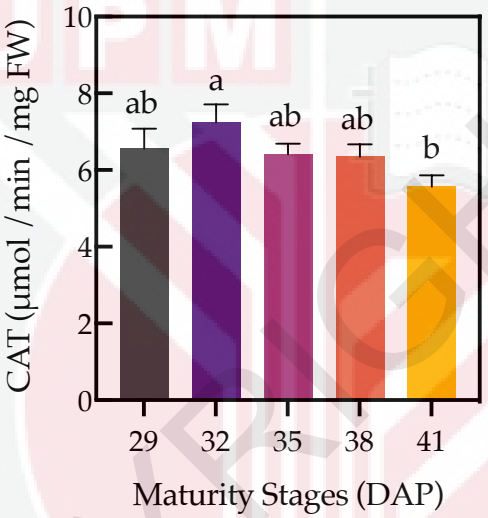

Figure 3.16: CAT activity of sweet corn seeds harvested at different maturity stages. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

Based on ANOVA in Appendix 3.22, significant interaction between maturity stages and drying temperature was observed in POD activity (Figure 3.17). POD amount increased reaching a peak at 38 DAP at 129.35 nmol /min /mg FW before it reduced. Drying at 40°C provides the highest CAT in exception of 29 and 41 DAP. The highest POD showed no significant difference at 35 and 38 DAP when both seeds were dried at 40°C while the rest is significantly lower with drying at 45°C giving the lowest at every maturity stage.

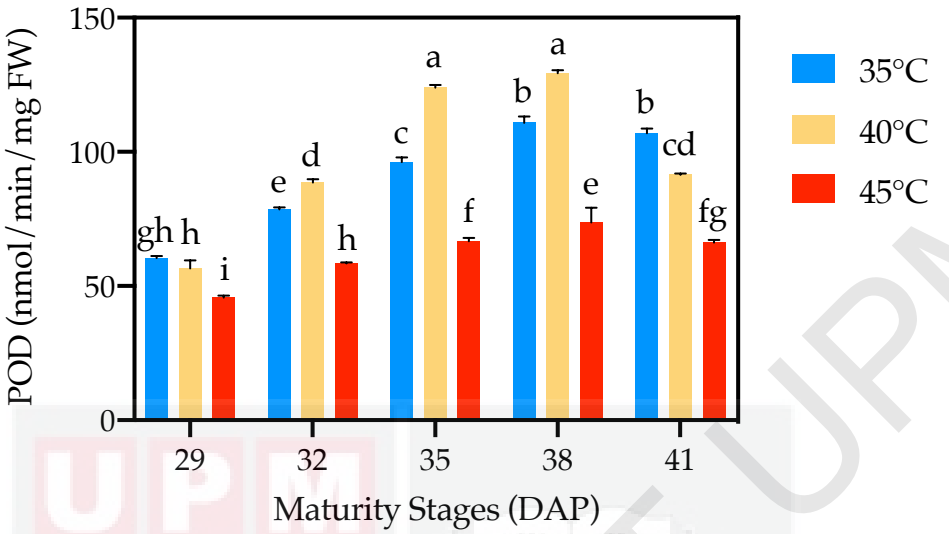

Figure 3.17: Interaction between seed maturity stages and different drying temperature on POD activity of sweet corn seeds. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

3.3.1.18 Malondialdehyde Content

Based on MDA content as in Figure 3.18 and Appendix 3.23, there was no interaction between maturity stages and drying temperature, but both factors were independently affecting the level of lipid peroxidation. Highest content of MDA was observed in early harvested seeds, 29 DAP (1.364 mmol /g FW) followed by 32 DAP at 1.1 mmol /g FW. Seeds that harvested between 35 to 41 DAP had the lowest MDA content and no significant difference was observed among them. Drying at 45°C produced highest content of MDA (1.12 mmol /g FW) while there was no significant difference between the seeds dried at 35°C and 40°C observed in MDA content.

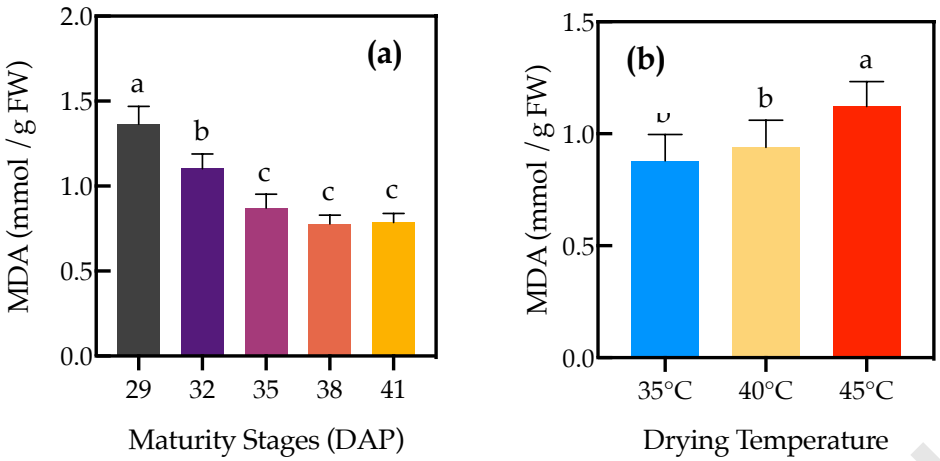

Figure 3.18: Lipid peroxidation measured in amount of malondialdehyde based on maturity stages (a) and drying temperature (b). Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

3.3.2 Seed Storability Performance

Seed quality and performance of sweet corn GSH11005Y for 12 months storage are as follows.

3.3.2.1 Final Germination Percentage

ANOVA summary of seed viability of sweet corn seed within a year storage is summarized in Table 3.2. Maturity stages and drying temperatures had no significant effect on seed viability up to 3 months after storage (MAS). However, at 6 MAS, drying temperature significantly influenced seed viability.

Table 3.2: Summary of seed viability based on final germination percentage of sweet corn collected at different maturity stages and dried at different drying temperature at three months intervals of a year storage.

Storage Period	Initial	3 Months	FGP (%) 6 Months	9 Months	12 Months
Maturity Stages	ns	ns	ns	ns	**
29 DAP	99.33	97.67	97.67	93.33	
32 DAP	99.00	98.00	99.67	95.53	
35 DAP	98.33	99.33	99.00	96.67	
38 DAP	99.33	99.33	98.33	96.67	
41 DAP	100	98.67	98.33	98.00	
Drying Temperature	ns	ns	**	**	**
35°C	99.4	99.00	99.4 a	97.8 a	
40°C	99.4	99.00	99.2 a	97.0 a	
45°C	98.8	97.80	97.2 b	93.2 b	
Maturity Stages × Drying Temperature	ns	ns	ns	ns	** (Refer Figure 3.19)

FGP is final germination percentage.
** indicates highly significant differences at P ≤ 0.0001.
ns indicates no significant differences.
means with same letter vertically in each of seed quality parameters are not significantly different at P > 0.05 using Tukey Test.

Germination remained high at 6 MAS, but seeds dried at 45°C exhibited the lowest viability at 97.2%, while seeds dried at 35°C and 40°C maintained high germination rates of 99.4% and 99.2%, respectively. Maturity stage did not have an effect at this time. At 9 MAS, the same pattern was observed as at 6 MAS.

There was no interaction between maturity stages and drying temperatures; however, drying temperature alone affected seed viability. Seeds dried at 45°C showed a decrease in viability to 93.2%, while seeds dried at 35°C and 40°C had significantly higher viability, at 97.8% and 97.0%, respectively. At 9 MAS, maturity stage continued to have no effect on seed viability.

At the end of one year of storage, a significant interaction between maturity stages and drying temperatures was observed (Figure 3.19). Seeds from all maturity stages dried at 35°C and 40°C showed similar high viability, but viability decreased when seeds were dried at 45°C. Germination peaked in seeds harvested between 32 and 38 days after planting (DAP) and dried at either 35°C or 40°C

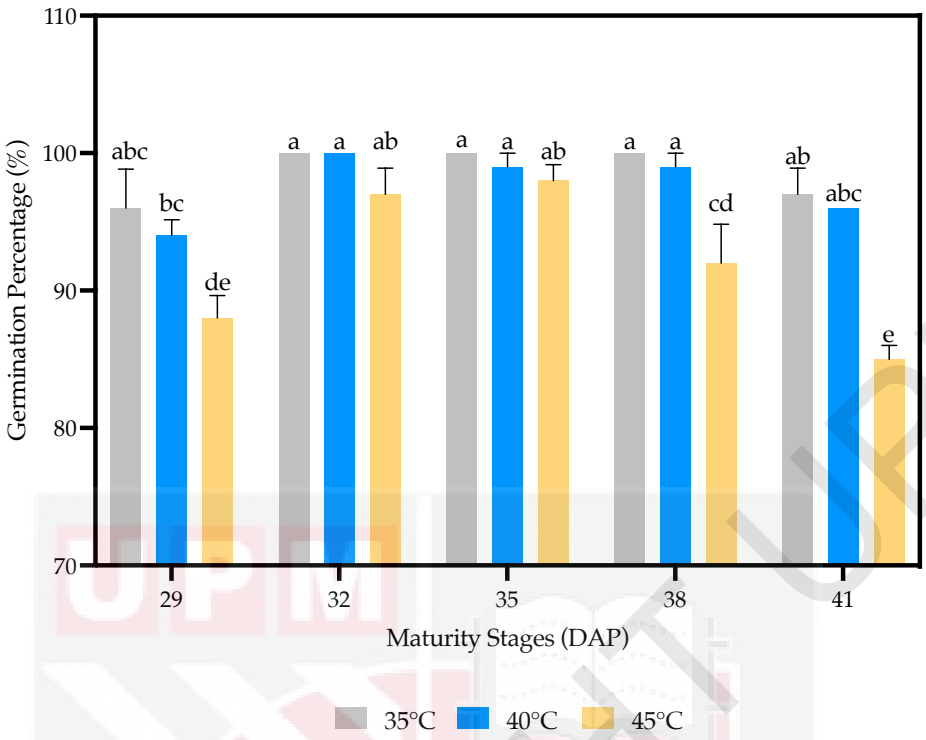

Figure 3.19: Final germination percentage of seeds harvested at different maturity stages and different drying temperature after 12 months in storage. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Tests.

3.3.2.2 Mean Germination Time

After 3 MAS, MGT of sweet corn was influenced by the maturity stages but not by the drying temperature, and there was no significant interaction between the two factors at any of the MAS observed. Seeds harvested earlier exhibited shorter MGT. For example, seeds harvested at 29 DAP took an average of 4.04 days to germinate, while seeds harvested at 32 and 35 DAP took 4.14 days and 4.16 days, respectively, with no significant differences observed between these three groups. In contrast, seeds harvested later showed a longer MGT, with seeds collected at 41 DAP taking the longest time

to germinate, at 4.48 days, which represents an increase of 10.9% compared to the seeds harvested at 29 DAP.

This trend continued at 6 and 9 MAS, where seed maturity stages were the only factor affecting MGT. At 6 MAS, seeds harvested between 29 and 35 DAP had a germination time between 4.15 and 4.20 days, while seeds harvested between 38 and 41 DAP took longer, ranging from 4.39 to 4.43 days, representing an increase of 5.8% to 6.9% in MGT. At 9 MAS, seeds harvested between 29 and 35 DAP took 4.21 to 4.24 days, while those harvested between 38 and 41 DAP took 4.41 to 4.42 days, with an increase of 4.7% to 5.1% compared to the earlier maturity stages.

At 12 MAS, however, neither maturity stage nor drying temperature had a significant effect on MGT, despite the previous effects observed at earlier storage times. Based on MGT, seed drying temperature did not affect germination time at any of the time points. The ANOVA summary for the MGT of sweet corn is shown in Table 3.3.

Table 3.3: Summary of seed vigour based on mean germination time of sweet corn collected at different maturity stages and dried at different drying temperature at three months intervals of a year storage.

Storage Period	Initial	3 Months	**MGT** (days) 6 Months	9 Months	12 Months
Maturity Stages	**	**	**	*	ns
29 DAP	3.71 b	4.04 c	4.15 b	4.21 b	3.71
32 DAP	3.72 b	4.14 bc	4.20 b	4.24 b	3.73
35 DAP	3.73 b	4.16 bc	4.19 b	4.21 b	3.73
38 DAP	3.96 a	4.34 ab	4.39 a	4.41 a	3.79
41 DAP	4.00 a	4.48 a	4.43 a	4.44 a	3.85
Drying Temperature	ns	ns	ns	ns	ns
35°C	3.86	4.29	4.35	4.32	3.74
40°C	3.80	4.14	4.21	4.23	3.68
45°C	3.82	4.24	4.25	4.40	3.86
Maturity Stages × Drying Temperature	ns	ns	ns	ns	ns

MGT is mean germination time.
** indicates highly significant differences at $P \leq 0.0001$.
* indicates significant differences at $P \leq 0.05$.
ns indicates no significant differences.
means with same letter vertically in each of sections are not significantly different at $P > 0.05$ using Tukey Test.

3.3.2.3 Germination Rate Index

ANOVA of GRI is summarized in Table 3.4. After 3 MAS, both maturity stages and drying temperature influenced seed vigour as measured by the GRI. However, no significant interaction was observed between the two factors throughout storage periods. Seeds harvested early exhibited the highest GRI. For example, seeds collected at 29 DAP had the highest GRI at 24.64, followed by seeds harvested at 32 DAP (24.36) and 35 DAP (23.99), with no significant differences observed among them. In contrast, seeds harvested later, particularly at 41 DAP, had significantly lower GRI at 22.45, representing a decrease of 9.0% compared to seeds harvested at 29 DAP. At 6 MAS, only maturity stage affected the GRI of sweet corn seeds. The same trend observed at 3 MAS continued, with seeds harvested between 29 and 35 DAP performing similarly and significantly better than seeds harvested between 38 and 41 DAP. The GRI for seeds harvested at 29 to 35 DAP ranged from 24.00 to 24.64, while seeds harvested at 38 to 41 DAP showed a lower GRI ranging from 22.45 to 23.00, a decrease of 5.7% to 9.0%. However, at 9 MAS, maturity stage no longer affected GRI, but drying temperature did. Seeds dried at 35°C (22.76) and 40°C (23.44) showed significantly better GRI compared to seeds dried at 45°C (21.72), which exhibited a decrease of 3.5% to 7.3% in GRI. At the end of one year of storage (12 MAS), neither maturity stage nor drying temperature significantly affected seed vigour in terms of GRI performance, indicating that the factors influencing seed vigour had diminished over time.

Table 3.4: Summary of seed vigour based on germination rate index of sweet corn collected at different maturity stages and dried at different drying temperature.

Storage Period	GRI				
	Initial	3 Months	6 Months	9 Months	12 Months
Maturity Stages	**	**	**	ns	ns
29 DAP	27.44 a	24.64 a	23.74 a	22.39	25.77
32 DAP	27.21 a	24.36 ab	23.99 a	22.85	27.42
35 DAP	26.84 ab	23.99 ab	23.92 a	23.20	25.99
38 DAP	25.58 bc	23.46 b	22.74 b	22.31	26.80
41 DAP	25.31 c	22.35 c	22.47 n	22.43	25.39
Drying Temperature	ns	*	ns	ns	ns
35°C	26.21	23.42 b	23.21	22.76	27.05
40°C	26.73	24.38 a	23.83	23.44	27.03
45°C	26.51	23.47 b	23.15	21.72	24.73
Maturity Stages × Drying Temperature	ns	ns	ns	ns	ns

GRI is germination rate index.
** indicates highly significant differences at $P \leq 0.0001$.
* indicates significant differences at $P \leq 0.05$.
ns indicates no significant differences.
means with same letter vertically in each of sections are not significantly different at $P > 0.05$ using Tukey Test.

3.3.2.4 Germination Index

As can be seen in Table 3.5, there was a significant effect of maturity stage on the seed vigour based on GI after 3 MAS. However, drying temperature did not affect GI and there was no interaction was observed between the two factors in all MAS observed. Same trend in GRI was observed in GI where the seeds harvested between 29 to 35 DAP performed equally well and better than the seeds collected late. For example, the seed harvested as early 29 DAP has a GI of 386.33, the best among treatments, while the seeds harvested according to industry standard was at 378.33 although there was no significant difference between the two. However, harvesting late at 41 DAP performed the lowest GI (347.33). This trend was observed even at six MAS where all seeds harvested between 29 to 35 DAP perform equally well and seeds harvested late performed poor based on GI. At 9 MAS, seeds GI was no longer affected by which maturity stages seeds were harvested. It shifted to the role of drying temperature. Seeds dried at 35°C (354.6) and 40°C (368.6) had better performance compared to seeds dried at 45°C (338.40). This was observed even after 12 MAS. Throughout the period, there was no interaction was observed based on seed GI.

Table 3.5: Summary of seed vigour based on germination index of sweet corn collected at different maturity stages and dried at different drying temperature.

Storage Period	GI Initial	3 Months	6 Months	9 Months	12 Months
Maturity Stages	**	**	**	ns	ns
29 DAP	426.00 a	386.33 a	376.63 a	351.00	398.33
32 DAP	423.00 a	383.00 ab	379.00 a	359.33	423.33
35 DAP	419.67 a	378.33 ab	378.33 a	366.00	403.33
38 DAP	401.00 b	367.00 b	355.33 b	347.00	418.00
41 DAP	400.00 b	347.33 c	350.33 b	349.00	395.00
Drying Temperature	ns	ns	ns	**	**
35°C	411.00	367.20	363.00	354.60 a	420.20 a
40°C	417.80	382.20	376.40	368.60 a	420.60 a
45°C	413.00	367.80	364.20	338.40 b	382.00 b
Maturity Stages × Drying Temperature	ns	ns	ns	ns	ns

GI is germination index.
** indicates highly significant differences at $P \leq 0.0001$.
* indicates significant differences at $P \leq 0.05$.
ns indicates no significant differences.
means with same letter vertically in each of sections are not significantly different at $P > 0.05$ using Tukey Test.

3.3.2.5 Coefficient Velocity of Germination

After 3 MAS, both maturity stages and drying temperature affected the CVG of sweet corn seeds, but there was no significant interaction between both factors was observed. Like other seed vigour parameters explained before, seeds harvested between 29 to 35 DAP produced faster seed germination compared the seeds harvested late between 38 to 41 DAP. In the 3 MAS, seeds harvested at 29 DAP has a value of 24.78 of CVG, the best among all maturity stages in that point of observation but it was still comparable with industry standard harvest (35DAP – 24.17). As expected, seeds harvested at 41 DAP performed the poorest with CVG value of 22.41 only. Based on drying temperature, seeds dried at 40°C (24.29) had the best CVG while seeds dried at 35°C (23.4) and 45°C (23.64) performed the lowest. However, at 6 and 9 MAS, only maturity stages affected the seeds CVG with the same trend as previous MAS was observed where the seeds collected early performed better than late harvested seeds. Details of the CVG of sixth and ninth MAS is illustrated in Table 3.6.

Table 3.6: Summary of seed vigour based on coefficient velocity of germination of sweet corn collected at different maturity stages and dried at different drying temperature.

Storage Period	CVG Initial	3 Months	6 Months	9 Months	12 Months
Maturity Stages	**	**	**	*	ns
29 DAP	27.01 a	24.78 a	24.14 a	23.58 a	27.33
32 DAP	26.89 a	24.19 ab	23.85 a	23.66 a	27.27
35 DAP	26.86 a	24.17 ab	23.96 a	23.77 a	27.17
38 DAP	25.34 b	23.32 bc	22.87 b	22.72 b	26.60
41 DAP	25.04 b	22.41 c	22.61 b	22.59 b	26.21
Drying Temperature	ns	*	ns	ns	ns
35°C	25.96	23.40 b	23.09	23.18	27.04
40°C	26.44	24.29 a	23.82	23.69	27.48
45°C	26.30	23.64 ab	23.55	22.93	26.22
Maturity Stages × Drying Temperature	ns	ns	ns	ns	ns

CVG is coefficient velocity of germination
** indicates highly significant differences at $P \leq 0.0001$.
* indicates significant differences at $P \leq 0.05$.
ns indicates no significant differences.
means with same letter vertically in each of sections are not significantly different at $P > 0.05$ using Tukey Test.

3.3.2.6 Seedling Vigour Index

As can be seen in Table 3.7, there was no interaction was observed in the 3 MAS of sweet corn based on SVI. However drying temperature influenced SVI in the 3, 6 and 9 MAS. A trend was observed where seed dried at 35°C and 40°C had better performance. For example, in 6 MAS, both seeds dried at 35°C (2838.27) and 4°0C (2858.95) had better performance in terms of SVI compared to seeds dried at 45°C (2673.04).

The same trend was observed during the 9 MAS with both seeds dried at 35°C and 40°C was superior in terms of SVI. At the end of 12 MAS, there was significant interaction was observed between maturity stages and drying temperature on sweet corn SVI and this is illustrated in Figure 3.20. From the figure, seeds dried at higher temperature had lower SVI and there was no significant difference for all maturity stages when the seeds dried between 35°C and 40°C.

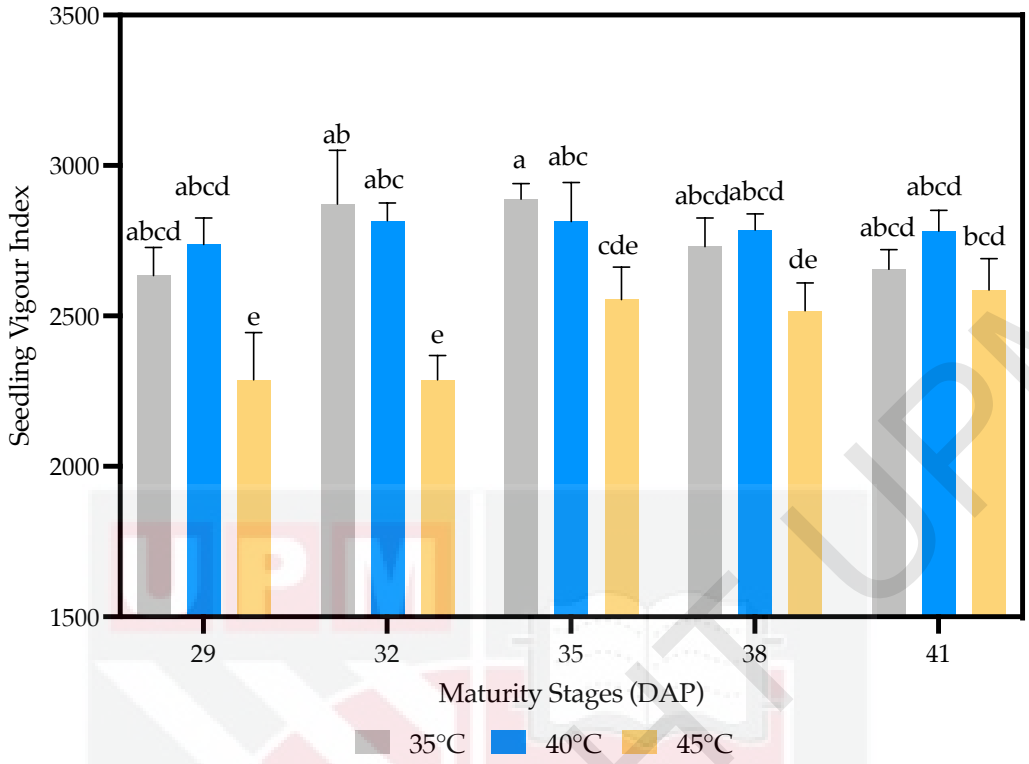

Figure 3.20: Seedling vigour index of seeds harvested at different maturity stages and different drying temperature after 12 months in storage. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Tests.

Table 3.7: Summary of seed vigour based on seedling vigour index of sweet corn collected at different maturity stages and dried at different drying temperature.

Storage Period	SVI				
	Initial	3 Months	6 Months	9 Months	12 Months
Maturity Stages	ns	ns	ns	ns	**
29 DAP	2,546.50	2,803.29	2,802.99	2,553.31	2,861.53 c
32 DAP	2,618.44	2,774.24	2,824.25	2,658.91	3,224.65 a
35 DAP	2,655.03	2,868.07	2,860.35	2,752.27	2,944.70 bc
38 DAP	2,632.96	2,736.59	2,753.58	2,677.89	3,183.57 a
41 DAP	2,641.79	2,673.89	2,709.27	2,674.55	3,100.85
Drying Temperature	*	*	**	**	**
35°C	2,623.04 ab	2,771.83 ab	2,838.27 a	2,755.93 a	3,125.05 a
40°C	2,680.56 a	2,839.39 a	2,858.95 a	2,787.39 a	3,175.34 a
45°C	2,553.22 b	2,702.38 b	2,673.04 b	2,446.83 b	2,888.80 b
Maturity Stages × Drying Temperature	ns	ns	ns	ns	* (Refer Figure 3.20)

SVI is seedling vigour index.
** indicates highly significant differences at $P \leq 0.0001$.
* indicates significant differences at $P \leq 0.05$.
ns indicates no significant differences.
means with same letter vertically in each of sections are not significantly different at $P > 0.05$ using Tukey Test.

3.3.2.7 Seedling Length

After 3 MAS, there was no interaction was observed between maturity stages and drying temperature on seedling length and can be referred to Figure 3.8. However, only drying temperature shown a significant difference when it comes to seedling length in the 3, 6 and 9 MAS. In 3 MAS, seedling length of seeds dried in 35°C and 40°C was higher than 45°C. Like many other parameters being explained before, seeds dried at 35°C (28.55 cm/ seedling) and 40°C (28.82 cm/seedling) had superior seed length in comparison with the seeds dried at 45°C (27.52 cm/seedling) at the end of 6 MAS and for seeds dried at 45°C it reduced to 26.21 cm per seedling. By the end of 12 MAS, maturity stages were observed to influence length of sweet corn seedling. Finding was interesting as the seeds harvested late had longer sweet corn seedling length compared to early harvested seed. For example, the seeds harvested at 41 DAP was around 32.63 cm /seedling while the seeds harvested at 29 DAP was only around 30.82cm /seedling. Seeds that were harvested at 32 DAP was around 31.17 cm /seedling. Summary of results of seedling length based on different maturity stages and drying temperature can be read in Table 3.8.

Table 3.8: Summary of seedling length of sweet corn collected at different maturity stages and dried at different drying temperature.

Storage Period	Seedling Length (cm /seedling)				
	Initial	3 Months	6 Months	9 Months	12 Months
Maturity Stages	ns	*	ns	ns	*
29 DAP	25.73	28.69 ab	28.71	27.29	30.82 c
32 DAP	26.46	28.29 abc	28.34	27.80	32.57 ab
35 DAP	27.01	28.88 a	28.83	28.44	31.17 bc
38 DAP	26.50	27.55 bc	28.00	27.69	32.06 abc
41 DAP	26.51	27.11 c	27.55	27.28	32.63 a
Drying Temperature	*	*	*	**	ns
35°C	26.51 ab	28.00 a	28.55 a	28.39 a	31.68
40°C	26.97 a	28.69 a	28.82 a	28.50 a	32.58
45°C	25.84 ab	27.63 b	27.52 b	26.21 b	31.28
Maturity Stages × Drying Temperature	ns	ns	ns	ns	ns

** indicates highly significant differences at $P \leq 0.0001$.
* indicates significant differences at $P \leq 0.05$.
ns indicates no significant differences.
means with same letter vertically in each of sections are not significantly different at $P > 0.05$ using Tukey Test.

3.3.2.8 Seedling Root Dry Weight

Based on the seedling root dry weight, there was no significant interaction was observed between maturity stages and drying temperature. However, at the end of 3 MAS, both factors, maturity stages and drying temperature and independently affecting seedling root dry weight with no significant interaction was observed between the two as can be seen in Table 3.9. Based on seedling root dry weight, the best performing seed was observed when the seeds harvesting late. For example, the heaviest root dry weight was obtained from 41 DAP seeds (0.0246 g /seedling). The lowest was observed when the seeds harvested at 29 DAP at (0.02 g /seedling). Seeds harvested at 35 DAP produced seedling with average root dry weight of 0.0207 g /seedling which is significantly not differed with 29 DAP. On the other hand, seeds were dried at 35°C (0.0239 g /seedling) had more mass compared to seeds dried at 40°C (0.0216 g /seedling) and 45°C (0.0205 g /seedling). For month six and nine and twelve, similar observation was made where seeds harvested late would produce heavier mass of root compared to the earlier harvested seeds. For example, the seeds harvested at 41 DAP was significantly higher compared to the rest with an average seedling root dry weight of 0.0286 g /seedling and dropped to 0.0233 g /seedling in ninth month and only 0.022 g /seedling and the end of storage period. However, the earliest harvested seeds, 29 DAP was consistently produced lower root dry weight from 0.0227 g /seedling (6 MAS) to 0.0199 g /seedling (9 MAS) and finally at 0.0173 g /seedling (12 MAS). In comparison, the seeds harvested at 35 DAP was average between the two and

detail of it can be found in in Table 3.9. Throughout 6 to 12 MAS, drying temperature only affected root dry weight at the end of storage period. Interestingly, the seeds dried at 40°C (0.022 g /seedling) had a superior seed dry weight compared to 35°C (0.201 g /seedling) and 45°C (0.019 g /seedling) in this study.

Table 3.9: Summary of seedling root dry weight of sweet corn collected at different maturity stages and dried at different drying temperature.

Storage Period	Seedling Root Dry Weight (g /seedling)				
	Initial	3 Months	6 Months	9 Months	12 Months
Maturity Stages	ns	**	**	*	**
29 DAP	0.032	0.0203 c	0.0227 c	0.0199 b	0.0173 c
32 DAP	0.0314	0.0216 bc	0.0259 ab	0.0231 a	0.0217 a
35 DAP	0.0371	0.0207 bc	0.0257 b	0.0239 a	0.0195 b
38 DAP	0.0357	0.0229 ab	0.0271 ab	0.0242 a	0.0217 a
41 DAP	0.0359	0.0246 a	0.0286 a	0.0233 a	0.0220 a
Drying Temperature	**	*	ns	ns	**
35°C	0.0349 ab	0.0239 a	0.0267	0.0226	0.0202 b
40°C	0.0374 a	0.0217 b	0.0265	0.0241	0.0221 a
45°C	0.0312 b	0.0205 b	0.0248	0.0219	0.0191 b
Maturity Stages × Drying Temperature	ns	ns	ns	ns	ns

** indicates highly significant differences at $P \leq 0.0001$.
* indicates significant differences at $P \leq 0.05$.
ns indicates no significant differences.
means with same letter vertically in each of sections are not significantly different at $P > 0.05$ using Tukey Test.

3.3.2.9 Seedling Shoot Dry Weight

In seedling shoot dry weight, there was no significant interaction was observed between the maturity stages and drying temperature throughout 12 MAS as can be seen in Table 3.10. There was no effect of both factors on seedling shoot dry weight up to 6 MAS. However, in the 9 MAS, both factors had an independent effect on this parameter. Same trend as root dry weight was observed in which seeds harvested late had better performance compared to early harvested seeds. For example, in the 9 MAS, seeds collected at 38 DAP has the highest seedling shoot dry weight at 0.0299 g/seedling compared to seeds harvested at 29 DAP where it only produced shoot weighing 0.0259 g /seedling. Finding at 12 MAS was quite interesting as seeds collected at 32-35 DAP showed the heaviest seedling shoot rather than the late harvested seeds. Seeds collected at 32 DAP produced shoot weighing 0.0339 g /seedling while the seeds collected at 41 DAP has shoot weight of 0.0298 g /seedling.

Table 3.10: Summary of seedling shoot dry weight of sweet corn collected at different maturity stages and dried at different drying temperature.

Storage Period	Seedling Shoot Dry Weight (g /seedling)				
	Initial	3 Months	6 Months	9 Months	12 Months
Maturity Stages	**	ns	ns	**	**
29 DAP	0.0228 b	0.0273	0.0257	0.0259 b	0.0298 b
32 DAP	0.0239 b	0.0282	0.0267	0.026 b	0.034 a
35 DAP	0.0257 a	0.0299	0.0281	0.027 b	0.0336 a
38 DAP	0.0258 a	0.0296	0.0262	0.0299 a	0.0332 b
41 DAP	0.0258 a	0.0286	0.0258	0.0278 ab	0.0298 b
Drying Temperature	*	ns	ns	**	ns
35°C	0.0253 a	0.0289	0.0264	0.0287 a	0.0333
40°C	0.0254 a	0.0295	0.0272	0.0277 a	0.0329
45°C	0.0237 b	0.0277	0.0255	0.0257 b	0.0324
Maturity Stages × Drying Temperature	ns	ns	ns	ns	ns

** indicates highly significant differences at P ≤ 0.0001.
* indicates significant differences at P ≤ 0.05.
ns indicates no significant differences.
means with same letter vertically in each of sections are not significantly different at P > 0.05 using Tukey Test.

3.4 Discussion

Quality seeds can be translated to have high viability and vigour and has superior seedling performance. All these traits varied depending on many factors such as maturity stages in which seeds are harvested and post-harvest activities such as drying (McDonald & Copeland, 2001). Physiological maturity is one of the indicator of when seed should be harvested. However, harvesting at this stage usually accompanied with higher moisture content, hence finding the balance of when to harvest is important (Bewley et al., 2013). Early harvesting may result in low quality such poor viability and storability, less vigour and can lead to poor seedling performance, while delaying harvest increase risk of loss of quality seeds (Leprince et al., 2017).

In this study, although seed fresh weight of GSH11005Y maximized at 32 DAP, during seed moisture measurement, it was observed that seed maximum dry weight pre drying was obtained at 29 DAP and dropped after that. This finding is different with Guan et al. (2013) where seed fresh weight of Shuyu No. 1 peaked at 26 DAP with dry weight accumulated maximized 38 DAP which noting that different varieties had different kernel development time, hence this kind of study needs to be repeated for every new varieties.

Seed moisture of the seed was high which is more than 40% for all maturity stages. Harvesting time in all of these studies should had the highest quality as study by Gupta et al. (2005) stated the moisture harvest that is more

between 40 to 50% in sweet corn had better germinability after storage which coincided with the moisture content observed in this study.

Pre-drying, both water soluble sugar, sucrose, and glucose content of GSH11005Y variety had a gradual reduction between 29 to 41 DAP while starch on the other hand had the optimum content at 32 DAP before slightly decreased. In comparison with Supersweet 2018, another *sh2* sweet corn variety had almost stagnant trend in starch content from 22 to 42 DAP when the sucrose content starts to decline as early as 22 DAP (Cao et al., 2008).

Low seed moisture is key to a long-term storage, with increase of moisture lead to higher respiration rate, leading to many deterioration activities, and reduction of moisture can be done using artificial drying such as exposing seed to higher temperature (Barrozo et al., 2014), hence the need of post-harvest drying in this study. Drying at the 40°C and 45°C had similar trend in reducing seed moisture but drying at 35°C took longer time. Both different maturity stages and drying temperature tested showed an effect on seed attributes which includes the final seed dry weight.

In initial performance measurement in this study, there was no interaction between maturity stages and drying temperature on seed viability of GSH11005Y with germination test were maximum with average of 99.17%. However, difference was observed on seed vigour as affected by different maturity stages. Despite higher seed leachate obtained in early harvested

seeds, parameters on seed vigour established different perspective. (Kader, 2005) stated that MGT and CVG is depicted as the time (in days) taken for a population of seed to germinate, with the low MGT and high CVG imply a faster germination is observed. Based on maturity stages factor, seeds harvested between 29 to 35 DAP had same performance and faster than the seeds harvested late (38-41 DAP) in terms of MGT and CVG. GRI on the other hand measures the percentage of germination every day and this reflects uniformity and rapidity of germination (Esechie, 1994) where the same observation made where 29 to 35 DAP seeds had equal performance and better than 38 to 41 DAP seeds.

According to Osuna et al. (2015), during seed germination, sucrose however can work as a signal, inducing the accumulation of trehalose-6-phosphate (T6P) compound that promotes plant growth during root and shoot development. This might explain the faster germination observed in early harvested seeds observed in this study. Seeds harvested between 29 to 35 DAP had faster rapid germination based on MGT, GRI and CVG and in line with the amount of sucrose in the seed where it was higher than late harvested seeds. Likewise, this only for seed germination event, while the starch content has more prominent role in seed storability.

Lower seed vigour is common in late harvested seeds and had been observed in many crops such as soybean (Thant et al., 2017) and hybrid rice (Wang et al., 2018). In a study conducted by Thant et al. (2017), delayed harvested

soybean had lower seed qualities and attributed to ROS accumulation within the seeds. Deleterious effect of ROS accumulation can be obviated with the presence of antioxidant enzymes such as CAT and POD. In this research, CAT activity was affected by when the seeds were harvested. The lowest CAT was observed in 41 DAP, the late harvested seeds. Kurek et al. (2019) stated that CAT activity decreased as the seed ageing goes, explaining the reduction in seed CAT activity in late harvested seeds, which supposedly resulting poor protective mechanism against ROS accumulation.

MDA is by product of the lipid peroxidation due to ROS accumulation. High MDA was observed to be high on damaged seeds such as in controlled deteriorated soybean (Min et al., 2017). In this study however, seeds harvested early had the highest amount of MDA compared to late harvested seeds. It is important to note that seeds harvested early had the highest amount of moisture initially. In wet condition, inefficient respiration can lead to ROS accumulation leading to higher peroxidation damage thus increasing damage on lipid (Bewley et al., 2013) observed in early harvested despite higher CAT activity.

Despite the above finding, harvesting seeds late affecting seed storability when factoring in drying temperature in measuring seed quality. In this study, seed viability was not affected by both factors, up to 9 MAS. However, interaction between maturity stages and drying temperature was observed at 12 MAS. Finding showed that seeds harvested late and dried at higher

temperature (45°C) had the lowest viability compared to early harvested seeds. Seeds dried at 45°C for all maturity stages had lesser viability compared to the seeds to one dried in either 35°C or 40°C. Apart from this study, in other crop such as soybean, late harvesting not only affecting seed quality during early stage but intensify once seeds being stored due to higher metabolic activity and injuries related to moisture content during post-harvest drying (Vergara et al., 2019).

In this study, post-harvest drying has no effect on seed viability and vigour during the early stages, but it affects seedling performance. Based on initial performance, drying at 35°C and 40°C had equal performance in SVI, and all seedling performance parameters. Although according to Siddique & Wright (2003) that temperature of 45°C can shorten time to dry and generally safe to dry seeds, finding in this study found that to dry GSH11005Y sweet corn seed in 45°C would result in poor seedling establishment, where the lowest SVI, shortest root length and poor root and shoot dry mass was observed. According Rosa et al. (2015), high temperature and long drying period reducing seed quality of orange seed quality, and in this study, drying 45°C was the limit especially when the seeds harvested late.

Study on *Phaseolus vulgaris* seeds showed that the seeds with higher moisture content had greater susceptibility towards the damage during drying (Scariot et al., 2017). However, it is important to note that the longer the seeds in the field, makes it vulnerable to other damaging factor such as pest and disease

attack which contribute to the decline in seed quality, therefore finding the balance of when to harvest and drying is the key to successful seed production.

Drying at higher temperature is always associated to damage of seeds such as internal crack, split seed coat and many others (Siddique & Wright, 2003). In Chapter 2, Section 2.1.4, we have discussed that low carbohydrate in endosperm, resulting severe shrinking in seeds of sweet corn, increasing damage on seed coat reducing seed quality (Gupta et al., 2005). This supported the finding in this study, where 45°C drying reduced seed quality and seedling performance due to damages by exposing seed to higher temperature causing higher damage on seed membrane.

On Spanish groundnut, seeds dried at higher temperature provides the fastest way to reduce seed moisture, however it leads to greater damage due to leak in seed membrane (Nautiyal, 2009). This was proved in this study where higher seed leachate obtained from sample seeds dried at 45°C, indicating higher crack on seed coat. Seed leachate is a measure to estimate seed membrane integrity resulting by seed deterioration (Moncaleano-Escandon et al., 2013). The higher number of leachates indicates lower seed vigour as the seed membrane integrity is damaged. It was reported by Jittanit et al. (2010) that seeds dried artificially with crack would show poor establishment, hence the poor SVI, and lower dry mass, as seen in this study.

Deleterious effect of drying at higher temperature also observed after six MAS where seeds dried at 45°C had lower viability compared to seeds dried at 35°C and 40°C. Several parameters such as GI, SVI, seedling length, root and shoot dry weight was affected by the drying temperature with 35°C and 45°C had an equal performance, supporting the finding by other scholars such as Guiscem et al. (2002) and Gupta et al. (2005).

Apart from seed leachate, lipid peroxidation can be measured to indicate the level of damage on seed membrane. In this study, high temperature (45°C) increased the damage on seed plasma membrane resulting in higher amount MDA in the seeds. Damage in seed membrane lipid due peroxidation as provided by the level of MDA causing increase in seed permeability and cellular damage as indicated in the amount of seed leachate in conductivity test (Bewley et al., 2013).

In this study, POD content of 40°C performed the highest of all maturity stages in exception of 41 DAP. Study by Huang et al. (2021) showed that there was increased in CAT and POD when the drying temperature increased and this due to stress reaction due to high temperature stress. Sharma et al. (2012) stated that apart of POD is widely known as enzyme that response to stress such as drought, salinity, and mineral toxicity. This study had shown an increase in POD at 40°C as effect of heat stress condition might be explaining the finding.

In this study, amount of glucose, sucrose and starch also were measured. Glucose content in sweet corn seeds were affected by harvesting time and drying temperature. In this study, it was observed that glucose content was high when the seeds subjected to drying at higher temperature. There are several studies had shown that glucose can have a negative effect on seed not only during development but also during germination. Exogenous glucose had caused delayed in seed germination of *Lotus japonicus* (Zhao et al., 2009) and *Oryza sativa* (Zhu et al., 2009). A high amount of glucose also induces ABA that caused delay in seed germination (Dekkers et al., 2004; Price et al., 2003). High temperatures were found to induce ABA biosynthesis thus affecting seed germination (Toh et al., 2008). Based on the glucose content in this study, it was higher when the seed dried at higher temperature (45°C). Seedling performance, particularly on seedling length, shoot and root dry weight and SVI showed lower performance when the seeds dried at 45°C while 35°C and 40°C which had lower amount of glucose, hence better seedling performance.

In literature review, we had learnt that sucrose and starch conversion can be affected by the temperature. Study on sucrose and starch conversion showed an increment in starch content and reduce sucrose when the seeds were placed up to 27°C (Bakry et al., 2015; Olsen et al., 1990; Simla et al., 2010). However, finding in this study was contradict and show an increment in sucrose content and reduction of starch content. Drying at 45°C had the highest reduction in starch content compared to 35°C and 40°C. According to Nelson & Pan (1995), the enzyme regulation in synthesis of starch in potato tuber is the same as in

corn. A study by Geigenberger et al. (1998) was conducted on changes in the enzyme activities when the potato tuber incubated at different temperature ranging between 19°C up until 37°C. The study showed that the activity of ADP-Glucose pyrophosphorylase (AGPase), one of the enzymes responsible in conversion of sucrose to starch increased when the temperature increased up until 30°C before drop was observed. This increment may explain the increment based on the study by Bakry et al. (2015), Olsen et al. (1990) and Simla et al. (2010) in which the AGPase could induce the synthesis of starch in the sweet corn seed resulting the increment of starch observed when it is placed at higher temperature. AGPase has been considered one the rate limiting factors in the synthesis of starch as it is needed for conversion into hexose and ADP-glucose (Preiss, 2018). The modulation of the AGPase activity can be tracked to three major factors: allosteric regulation, thermal inactivation, and reductive activation. 3-phosphogylceric acid (3PGA) is the allosteric regulators promoting the activity of AGPase (Boehlein et al., 2008). In the study earlier by Geigenberger et al. (1998), the finding also showed the activity of 3PGA increased when the temperature increased and peaking around 25°C. This resulted in higher level ADP-glucose as observed in 25°C compared to 19°C in potato tuber increasing the starch synthesis.

3.5 Conclusion

Considering all the findings, for immediate use, GSH11005Y, a *sh*$_2$ sweet corn seed can be harvested as early as 29 DAP as it has high viability, vigour, and seedling performance. However, for long term storage, seed should be harvested at 35 DAP as seed quality collected in this stage had optimum stability in terms of viability and vigour throughout 12 MAS.

Above recommendation is based on initial performance where seed maturity did not affect seed viability but has a pronounced effect on seed vigour where seeds harvested between 29 to 35 DAP had better quality compared to late harvested seed (38 to 41 DAP). However, during storage period, 32-38 DAP was more consistent in seed viability. Apart from that, seed vigour was also higher in 29-35 DAP seed especially in MGT and CVG. Based on the study, there was reduction in starch content after drying, but lesser reduction was observed seeds in early harvested seeds (29-35 DAP) compared to late harvested seeds (38-41 DAP). Therefore, finding the right balance between seed viability and vigour while maintaining higher starch amount, all in initial performance and storage is important, hence, 35 DAP seeds sit right in a sweet spot of quality seed.

To reduce seed moisture, it is recommended that seeds are to be dried at 40°C as it gives the shorter time taken to obtain desired moisture content for storage, and the same time maintaining seed viability, vigour, and seedling

performance. Post-harvest drying temperature affect seed starch content with higher reduction observed when seed subjected to 45°C while drying at 35°C and 40°C was comparable in starch content after drying. Sucrose content remain high in early harvested seed, accompanied with lesser reduction in starch, accompanied by higher CAT activity may aid seed germination.

In terms of seedling performance, drying temperature plays an important roles in seedling establishment such as dry mass, seedling length and SVI. Drying at 35°C and 40°C had an equal performance, but time needed to reduce seed moisture was better in 40°C. Drying at 45°C provides the fastest way to lower seed moisture, however it had a detrimental effect on seedling performance with higher seed leachate indicating the damage after exposing to higher temperature. During the 12 MAS, this trend was observed consistently indicating the best way to dry sweet seed is to dry it at 35°C and 40°C with drying at 40°C had its leverage as it takes shorter time to dry seed to 8% while maintaining seed quality. Higher POD activity to alleviate detrimental effect of ROS accumulation also was observed in the seeds dried at this temperature, contributing to its better storability.

CHAPTER 4

SEED LONGEVITY IMPROVEMENT THROUGH VACUUM PACKAGING AND OPTIMIZATION OF STORAGE TEMPERATURE IN SWEET CORN

4.1 Introduction

Deterioration in seeds include many progressive detrimental changes as the seeds undergo ageing (Bewley et al., 2013). As discussed before, seed ageing in sweet corn is very prominent during storage. For example, sweet corn hybrids D657 and D769 germination from Brazil that had germination reduced from 100% to between 45 to 60% in four months and completely not viable after eight months even in airtight condition. In Section 2.2 of Chapter 2, numerous factors that influenced seed deterioration was discussed.

To date, seeds are stored at low moisture as well as in cool temperature under low relative humidity condition to prolong its storability (Verdier et al., 2019). In companies such as GWG Sdn. Bhd, sweet corn seeds are stored in 16˚C to maintain seed longevity. However, seed quality can be reduced tremendously in the event where appropriate temperature storage is not used especially when cool room storage in unavailable (Fu et al., 2018).

Apart from the temperature, there is one factor that is hardly considered in the practice of storing seeds either for commercial or gene bank storage, which is oxygen (Groot et al., 2012) where it regulates many ageing physiological processes caused by respiration (Rajjou & Debeaujon, 2008). The biggest component of seed deterioration is mainly due to accumulation of reactive oxygen species (ROS) causing oxidative damage to seed cells (Wang et al., 2015). This includes superoxide (O_2^-), singlet oxygen ($^1O_2$), H_2O_2, OH^- which trigger numerous detrimental events including lipid peroxidation (Sharma et al., 2012). The increase of oxygen level in packaging has been associated to depletion of protective antioxidant mechanism. This includes enzymes such as SOD, CAT and POD (Wilson & McDonald, 1986).

Currently industry implemented a practise to keep seeds in air-tight packaging using either polyethylene bag or metalized polyethylene bag, providing a hermetic condition such as in Figure 2.5 in Chapter 2. The use of low oxygen atmosphere in packing system was done hermetically before and been proven to be a success to slow down the rate of deterioration when the seeds are stored in cool temperature. However, any potential enhancements in seed packaging should be explored, as they could lead to improved outcomes, particularly in terms of seed longevity.

Vacuum packaging is a process of sealing any products in a very low air level by evacuating air from the package using different air pressure which has been efficaciously slowing down ageing process in food industry, and some

research demonstrated promising result in maintaining seed quality such as in sunflower (Abreu et al., 2013), rye (Barzali et al., 2005), groundnut (Sastry et al., 2007), chilli (Deepa et al., 2013) and bitter gourd (Yeh et al., 2005). These studies showed an association with the elevation of antioxidant mechanism in those seeds.

Wang et al. (2018) demonstrated the possibility of storing the seed in ambient room temperature combined with hermetic condition. In this study, rice seeds were stored under vacuum condition, and it was observed that increasing storage temperature to ambient condition did not affect seed viability after the 60 days within storage. Another study on castor bean showed that the seed stored in vacuum condition had the highest germination and seedling emergence at 75% compared to airtight packing using plastic bag (65%) and paper bag (53%), all in room temperature after 12 MAS (Santos et al., 2016).

Therefore, based on the understanding of seed deterioration, and the possibility of providing alternatives to store sweet corn seeds, second study in this thesis was conducted to determine the effects of vacuum packaging and storage at different temperature on the longevity and quality of sweet corn seeds. In this chapter, the effects such as seed viability, seed vigour and seedling performance as well certain biochemical components especially on antioxidant mechanism were measured and discussed.

4.2 Materials and Methods

4.2.1 Seed Preparation, Packaging and Storage

In this study, sweet corn seeds of GSH11005Y were harvested at 35 DAP based on industry common practices for commercial seed production. Seed germination test was conducted to determine initial seed quality before seeds were placed into three types of packaging: full vacuum (VC), partial vacuum (PV), and airtight-sealed(ATS) condition, all in aluminium polyethylene (PE) bag. FV and PV was attained using a vacuum sealer machine at Seed Quality Laboratory in MARDI, Serdang, Selangor (2.979767,101.690203). To provide a FV condition, air pressure was set at -0.09 to -0.1 mPa while in PV condition, it was set between -0.07 to -0.08 mPa. Air pressure in ATS packaging was set between 0 to -0.01 mPa.

Seeds were then stored at 26±3°C room and 16°C cool room for 12 months. Data on storage temperature and relative humidity is presented in Appendix 4.1 and Appendix 4.2. Data on seed viability, seed vigour, seedling performance and biochemical components were measured every three months.

4.2.2 Seed Germination Test and Measurement of SeedVigour

Seed germination test was conducted using the sand method (ISTA, 2017). Procedures of seed germination test was conducted as described in Section 3.2.8 in Chapter 3.

4.2.2.1 Final Germination Percentage

FGP was determined based on Section 3.2.8.1 in Chapter 3.

4.2.2.2 Mean Germination Time

MGT was determined based on Section 3.2.8.2 in Chapter 3.

4.2.2.3 Germination Rate Index

GRI was determined based on Section 3.2.8.3 in Chapter 3.

4.2.2.4 Germination Index

GI was determined based on Section 3.2.8.4 in Chapter 3.

4.2.2.5 Coefficient Velocity of Germination

CVG was determined based on Section 3.2.8.5 in Chapter 3.

4.2.2.6 Seedling Vigour Index

SVI was determined using method in Section 3.2.8.6 in Chapter 3.

4.2.2.7 Measurement of Seedling Length

Measurement of seedling length was determined based on Section 3.2.8.7 in Chapter 3.

4.2.2.8 Measurement of Shoot and Root Dry Weight

Measurement of shoot and root dry weight was determined using method in Section 3.2.8.8 in Chapter 3.

4.2.3 Determination and Assay of Antioxidant Enzymes

Preparation of seed sample and enzyme extracts was prepared using method described in Section 3.2.11 in Chapter 3.

4.2.3.1 Measurement of Catalase (EC 1.11.1.6)

Measurement and assay of CAT was conducted based on the protocol described in Section 3.2.11.1 in Chapter 3.

4.2.3.2 Measurement of Guaiacol Peroxidase (EC 1.11.1.7)

Measurement and assay of POD was conducted based on the protocol described in Section 3.2.11.2 in Chapter 3.

4.2.3.3 Measurement of Superoxide Dismutase (EC 1.15.1.1)

SOD was measured using the method of Gupta et al. (1993). Supernatant from POD procedure was used in which 0.05 mL of it was added to a mixture of 1.5 mL of 100 mM potassium phosphate buffer, 0.1 mL of 3 mM EDTA, 0.1 mL of 200 mM methionine, 0.01 mL of 2.25 mM nitro-blue tetrazolium (NBT) and 1 mL of distilled water. Finally, 0.1 mL of 60 µM riboflavin was added to the reaction mixture in the dark. It was then placed under light (30 cm away from the source) of 15-watt florescent lamp for 15 minutes to allow colorimetric reaction. After 15 minutes under light, it was immediately covered using aluminium foil to stop the reaction. The mixture without enzyme was used as a control (placement together with sample under the light) while the mixture without enzyme (no placement under light) was used as a blanks. SOD activity

was measured using spectrophotometer at 560 nm and calculated using the following formula:

$$SOD = \frac{(\Delta A560nm\ control - \ \Delta A560nm\ sample) \times 100}{\Delta A560nm\ control}$$

$$SOD\ (unit/ml) = \frac{\%\ inhibation\ \times total\ volume}{50\ \times\ enzyme\ volume}$$

$$SOD\ (unit/mg\ FW) = \frac{unit/ml}{mg/ml\ enzyme}$$

4.2.4 Determination and Assay of Malondialdehyde

Measurement and assay of MDA was conducted based on the protocol described in Section 3.2.12 in Chapter 3.

4.2.5 Experimental Design and Data Analysis

There were two factors examined in this study. The first factor was packaging types, and the second factor was the storage temperature during seed storage and experiment was conducted in two-way factorial CRD, with four replications. Measurement of parameters were conducted at three months interval for a year.

ANOVA were carried out for all parameters and means comparison was done using Tukey Test with a minimum of 95% confidence interval. All statistical analysis will be carried out using statistical software, Statistical Analysis System (SAS 9.4).

4.3 Results

Seed performance based on seed quality, seedling performance and biochemical activities are as follows:

4.3.1 Seed Viability

ANOVA of final gemination percentage was summarized to Table 4.1. There was no significant interaction between packaging types and temperature on the seed viability of sweet corn at 3 MAS. Neither was there any significant difference on the single factor effect. However, the treatments started to show a significant effect on the seed viability at 6 MAS. Seeds that were stored in FV condition (FGP = 88.5%) and PV (FGP = 80.5%) performed better compared to air-tight sealed packaging with only 71.5% germination. Interestingly, up to 6 MAS, temperature had no effect on seed viability. In the 9 and 12 MAS, there was an interaction between packaging types and temperature on seed viability of sweet corn.

Table 4.1: Summary of analysis of variance on final germination percentage of sweet corn stored in different packaging types and stored in two different temperatures at three months intervals for year storage.

Final Germination Percentage (%)				
Source of Variation	**Storage Period**			
	3 Months	**6 Months**	**9 Months**	**12 Months**
Packaging Type	ns	**	*	**
Full Vacuum	90.50	88.50 a	72.50 a	72.00 a
Partial Vacuum	91.50	80.50 a	71.00 a	56.00 a
Air-tight Sealed	86.50	68.50 b	64.00 b	50.00 b
Storage Temperature	ns	ns	**	**
16°C	90.67	80.33	85.67 A	85.33 A
26±3°C	88.33	78.00	52.67 B	33.33 B
Packaging Type × Storage Temperature	ns	ns	* (Refer Figure 4.1)	* (Refer Figure 4.2)

** indicates highly significant differences at P ≤ 0.0001.
* indicates a significant differences at P ≤ 0.05.
ns indicates no significant differences.
means with same letter vertically in each of source of variation row sections are not significantly different at P > 0.05 using Tukey Test.

During the 9 MAS in Figure 4.1, seeds that were stored at 16°C maintain seed viability the best as observed in FV condition at 94% followed by the seeds in PV (88%). Seeds stored in air-tight sealed packaging was significantly lower at only around 75% viability. Storing seed in 26±3°C room temperature however has reduced germination compared to 16°C but it was not affected by packaging types as there was no significant difference among them ranging between 51 to 54%.

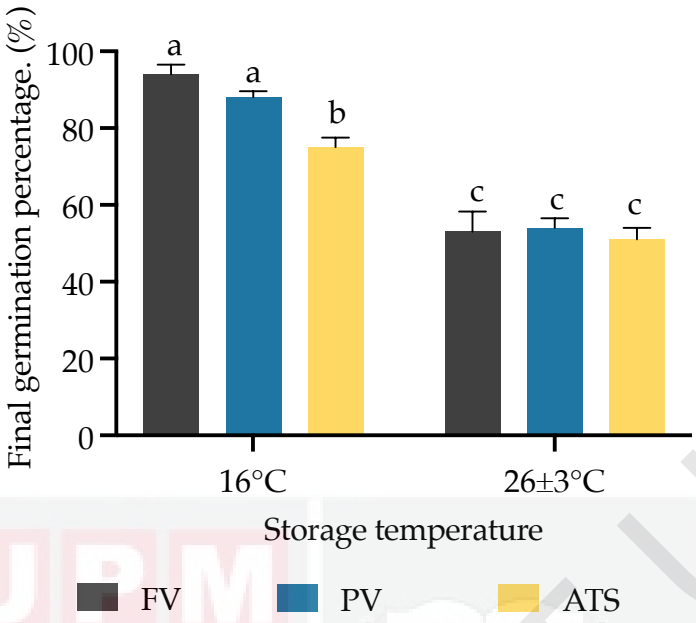

Figure 4.1: Final germination percentage after 9 months in storage. FV is full vacuum, PV is partial vacuum while ATS is air-tight sealed packaging. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

As can be seen in Figure 4.2, same trend was observed whereby seed viability maintained very high when the seeds were stored in 16°C with both vacuum and PV having the best FGP. At the end of the storage period, the highest seed viability was observed in seeds stored in FV packaging, at 92%, followed by seeds stored in PV packaging (86%). Seeds stored in ATS packaging had the lowest viability at 78%, significantly lower than FV and PV. Storage at 26±3°C room temperature resulted in reduced seed viability across all packaging types. Seeds stored in FV packaging maintained the best viability at 52% from 9 to 12 MAS, while seeds stored in PV and ATS packaging at 26±3°C showed a substantial decline in viability, with only 26% and 22% viability, respectively, which were the lowest values observed.

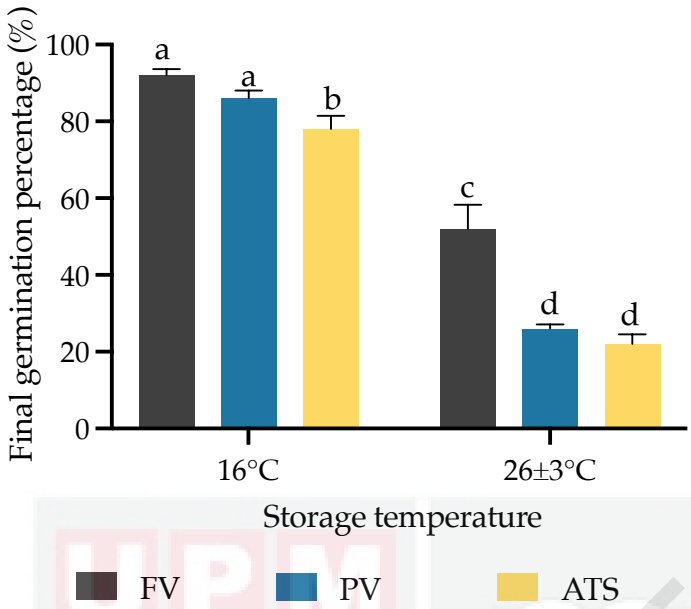

Figure 4.2: Final germination percentage at 12 Months. FV is full vacuum, PV is partial vacuum while ATS is air-tight sealed packaging. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

4.3.2 Mean Germination Time

Table 4.2 showed summary of result of MGT for 12 months. Same with seed viability, seed vigour based on MGT was not affected by either packaging types or storage temperature at 3 MAS. By 6 MAS, both factors independently affected seed MGT. Based on packaging types, seeds that were stored in FV condition (4.00 days) was superior compared to PV (4.06 days) and ATS (4.33 days) packaging while the seeds stored in 16°C (4.03 days) was significantly faster compared to 26±3°C room temperature (4.23 days).

Table 4.2: Summary of seed vigour based on mean germination time of sweet corn stored in different packaging type and stored in two different storage temperature at three months intervals of a year storage.

Mean germination time(days)				
Source of Variation	**Storage Period**			
	3 Months	**6 Months**	**9 Months**	**12 Months**
Packaging Type	ns	*	ns	**
Full Vacuum	4.30	4.00 a	4.82	5.00 a
Partial Vacuum	4.25	4.06 b	4.86	5.68 a
Air-tight Sealed	4.21	4.33 b	4.92	5.69 b
Storage Temperature	ns	*	**	**
16°C	4.22	4.03 B	4.21 B	5.21 B
26±3°C	4.30	4.23 A	5.53 A	5.69 A
Packaging Type × Storage Temperature	ns	ns	ns	** (Refer Figure 4.3)

** indicates highly significant differences at P ≤ 0.0001.
* indicates a significant differences at P ≤ 0.05.
ns indicates no significant differences.
means with same letter vertically in each of source of variation row sections are not significantly different at P > 0.05 using Tukey Test.

However, in the 9 MAS, only temperature showed effects on seeds MGT where the same trend in the earlier month was observed but the number of days was longer. Seeds stored in 16°C took 4.21 days while the seeds stored in 26±3°C room temperature took 5.53 days.

At the end of the storage study, there was a significant interaction between packaging types and storage temperature on seed's MGT. Seeds stored in flexible vacuum (FV) packaging exhibited the shortest MGT of 4.68 days, which was significantly lower (by 12.3%) compared to all other treatments. Notably, seeds stored in PV packaging at 16°C showed similar performance to those stored in FV packaging at room temperature (26±3°C), with MGTs of

5.32 days and 5.22 days, respectively. These values were significantly better (by 7.5% to 8.5%) than the seeds stored in air-tight sealed (ATS) packaging at 16°C, which had the longest MGT of 5.75 days. As shown in Figure 4.3, seeds stored in ATS packaging, regardless of temperature, consistently performed the poorest at the end of storage.

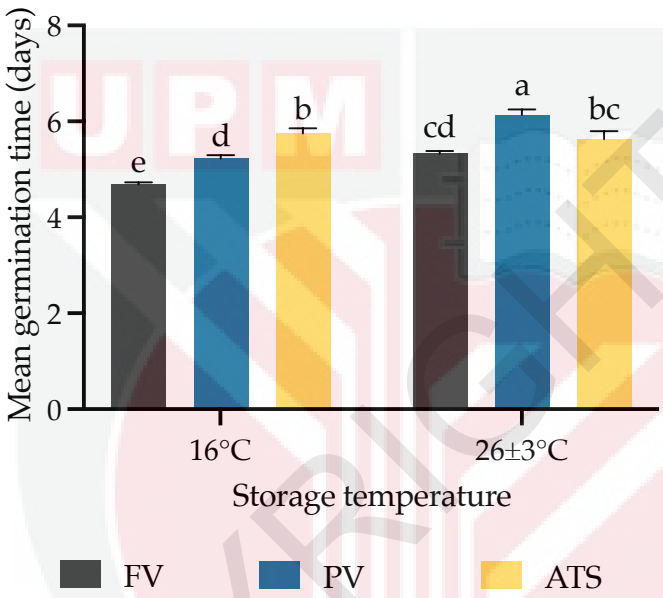

Figure 4.3: Mean germination time at 12 months after storage. FV is full vacuum, PV is partial vacuum while ATS is air-tight sealed packaging. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

4.3.3 Germination Index

GI of stored sweet corn was summarized in Table 4.3. At 3 MAS, there was no significant effect of packaging types and storage temperature on the GI of stored sweet corn seed. However, at 6 MAS, it was observed that packaging types influencing the GI but not the storage temperature. Seeds that were

stored in FV (353.5) has the highest GI was significantly better than the rest, this was followed by seeds stored in PV (316.5) and the lowest quality was observed in seeds stored in ATS packaging (215.5).

Table 4.3: Summary of seed vigour based on germination index of sweet corn stored in different packaging type and stored in two different storage temperature at three months intervals of a year storage.

Source of Variation	Germination index			
	Storage Period			
	3 Months	6 Months	9 Months	12 Months
Packaging Type	ns	**	**	**
Full Vacuum	334.50	353.50 a	244.50 a	222.50 a
Partial Vacuum	334.50	316.50 b	233.50 a	144.00 b
Air-tight Sealed	327.00	251.50 c	204.50 b	113.50 c
Storage Temperature	ns	ns	**	**
16°C	343.00	319.00	325.00 A	239.67 A
26±3°C	327.00	295.33	130.00 B	80.33 B
Packaging Type × Storage Temperature	ns	ns	** (Refer Figure 4.4)	** (Refer Figure 4.5)

** indicates highly significant differences at P ≤ 0.0001.
* indicates a significant differences at P ≤ 0.05.
ns indicates no significant differences.
means with same letter vertically in each of source of variation row sections are not significantly different at P > 0.05 using Tukey Test.

Interaction seeds stored at 9 and 12 MAS as summarized in Figure 4.4 and Figure 4.5. At 9 MAS, seeds stored at 16°C consistently outperformed those stored at 26±3°C, with significant differences observed across the three packaging types. The best GI was recorded in FV packaging at 16°C, with a GI of 361, followed by PV packaging (331) and ATS packaging (283). Seeds stored at 26±3°C, regardless of packaging type, showed a marked decrease in GI, ranging from 126 to 136, with no significant difference between packaging

types. This trend continued at 12 MAS, where seeds stored at 16°C again exhibited better performance compared to those stored at 26±3°C. Notably, seeds stored in ATS packaging at 16°C performed better than those in FV or PV packaging at the same temperature, but still showed a decrease of 9% to 21% compared to FV at 16°C. At 26±3°C, seeds stored in FV packaging performed better than those in PV and ATS, but their GI was still lower by 25% to 30% compared to seeds stored at 16°C. Interestingly, seeds stored in PV packaging performed similarly to those stored in ATS, with no significant difference between the two factors.

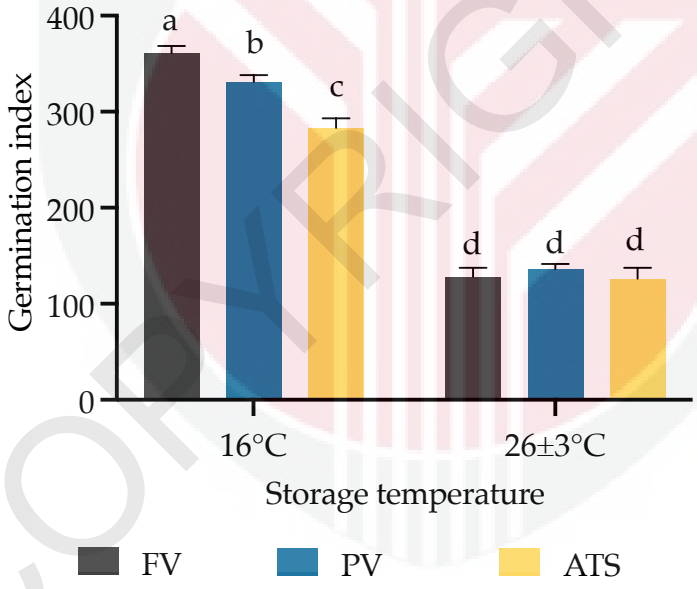

Figure 4.4: Interaction between packaging types and storage temperature on germination index of seeds stored at 9 months. FV is full vacuum, PV is partial vacuum while ATS is air-tight sealed packaging. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

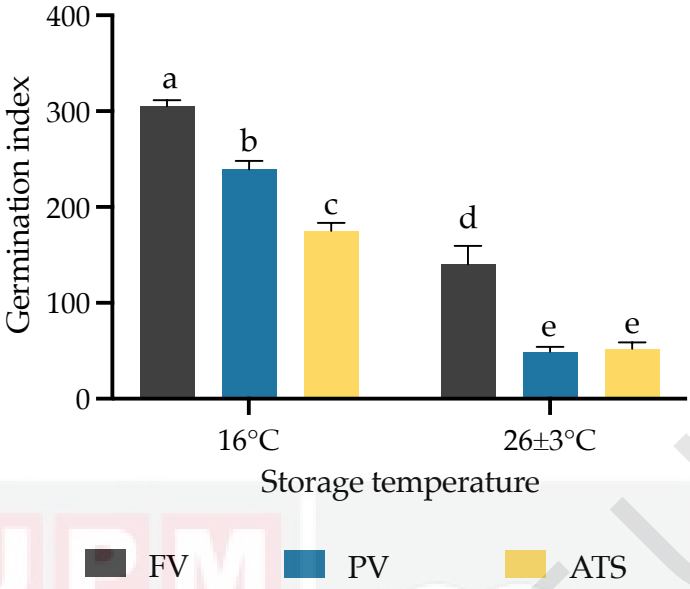

Figure 4.5: Interaction between packaging types and storage temperature on germination index of seed at 12 months. FV is full vacuum, PV is partial vacuum while ATS is air-tight sealed packaging. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

4.3.4 Germination Rate Index

Based on ANOVA in Table 4.4, both factors, packaging types and storage temperature had no significant interaction and effect on seed vigour based in GRI after 3 MAS. After 3 MAS, packaging started to show an effect on seed GRI with the seed in full packaging had the highest GRI at 22.66 and significantly better than PV (20.18) and the lowest at 16.25 for ATS packaging.

Table 4.4: Summary of seed vigour based on germination rate index of sweet corn stored in different packaging type and stored in two different storage temperature at three months intervals of a year storage.

Source of Variation	Germination rate index			
	Storage Period			
	3 Months	6 Months	9 Months	12 Months
Packaging Type	ns	**	**	**
Full Vacuum	21.39	22.66 a	16.14 a	15.11 a
Partial Vacuum	21.78	20.18 b	15.51 a	10.54 b
Air-tight Sealed	20.71	16.25 c	13.79 b	8.95 c
Storage Temperature	ns	ns	**	**
16°C	21.73	20.43	20.69 A	16.96 A
26±3°C	20.86	18.94	9.69 B	6.13 B
Packaging Type × Storage Temperature	ns	ns	** (Refer Figure 4.6)	ns

** indicates highly significant differences at P ≤ 0.0001.
* indicates a significant differences at P ≤ 0.05.
ns indicates no significant differences.
means with same letter vertically in each of source of variation row sections are not significantly different at P > 0.05 using Tukey Test.

After 9 MAS, there was a significant interaction was observed between packaging types and storage temperature as in Figure 4.6. The highest GRI was observed in FV at 22.82 followed by PV (21.07) and ATS packaging (17.95), all in 16°C cool room storage with significant difference was observed in all of them. However, all seeds in 26±3°C room temperature had the lowest GRI despite different packaging types, ranging from 9.44 to 10.01.

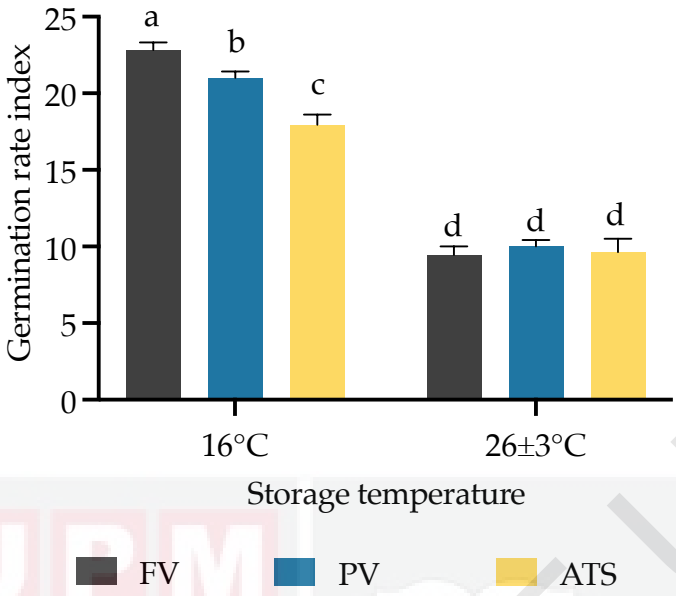

Figure 4.6: Interaction between packaging types and storage temperature on germination rate index at 9 months. FV is full vacuum, PV is partial vacuum while ATS is air-tight sealed packaging. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

At the end of 12 MAS, both packaging type and storage temperature independently affected seed's GRI. Seeds stored in FV packaging had the highest GRI, with a value of 15.11, followed by seeds stored in PV packaging (10.54) and the lowest GRI observed in ATS packaging (8.95). The GRI in FV packaging was 43.4% higher than PV and 68.1% higher than ATS. Regarding storage temperature, seeds stored at 16°C performed significantly better, with a GRI of 16.96, more than double the GRI of seeds stored at 26±3°C, which was 7.89.

4.3.5 Coefficient Velocity of Germination

Based on ANOVA conducted, there was no significant effect of both factors on the seed CVG up to 3 MAS. At 6 MAS, both factors, packaging types and storage temperature independently affecting the seed CVG with no interaction observed. As seen in Table 4.5, in 6 MAS, seeds stored in FV had the highest CVG at 25.08 followed by PV (24.64) and ATS (23.18) packaging with all treatment significantly differed to one another. Meanwhile, seeds stored in 16°C had better CVG at 24.85 compared to 26±3°C room temperature (23.75). However, at 6 MAS, only temperature environment influenced seed's CVG with same trend was observed where the seeds stored in 16°C had better performance than 26±3°C room temperature seeds.

Table 4.5: Summary of seed vigour based on coefficient velocity of germination corn stored in different packaging type and stored in two different storage temperature at three months intervals of a year storage.

Coefficient velocity of germination				
Source of Variation	**Storage Period**			
	3 Months	**6 Months**	**9 Months**	**12 Months**
Packaging Type	ns	*	ns	**
Full Vacuum	23.34	25.08	21.13	20.07 a
Partial Vacuum	23.61	24.64	20.94	17.75 b
Air-tight Sealed	23.75	23.18	20.75	17.61 b
Storage Temperature	ns	*	**	**
16°C	23.78	24.85 A	23.78 A	19.31 A
26±3°C	23.36	23.75 B	18.10 B	17.64 B
Packaging Type × Storage Temperature	ns	ns	ns	** (Refer Figure 4.7)

** indicates highly significant differences at P ≤ 0.0001.
* indicates a significant differences at P ≤ 0.05.
ns indicates no significant differences.
means with same letter vertically in each of source of variation row sections are not significantly different at P > 0.05 using Tukey Test.

At the end of 12 MAS, there was interaction was observed between packaging types and storage temperature on the seed's CVG and this was summarized in Figure 4.7. Seeds stored in FV in 16°C room had the best performance based on CVG. Interestingly, the seeds stored in FV packaging, despite being stored in 26±3°C room temperature, it had same performance with the seeds in PV and performed better than ATS packaging both that were stored in 16°C.

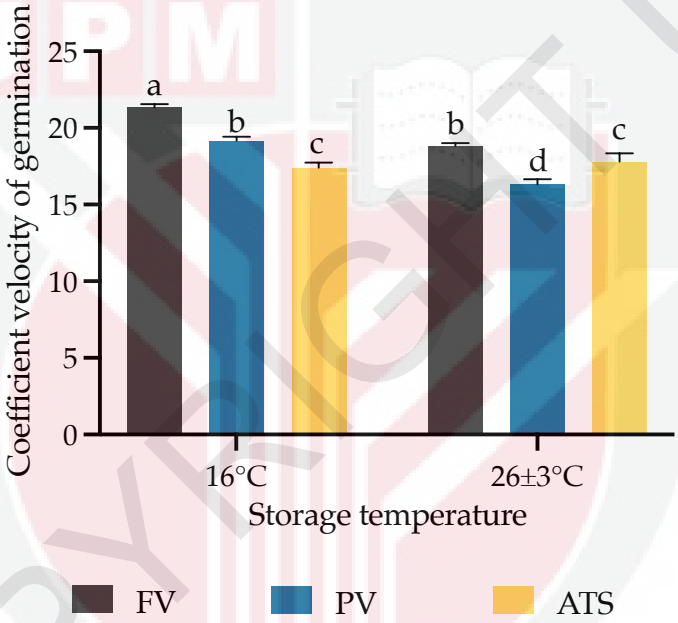

Figure 4.7: Seed's coefficient velocity of germination as affected by packaging types and storage temperature at 12 months storage. FV is full vacuum, PV is partial vacuum while ATS is air-tight sealed packaging. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

4.3.6 Seedling Vigour Index

SVI was not significantly affected by both packaging types and storage temperature at 3 MAS. The effects were only observed after 6 MAS with packaging types affecting the SVI. As can be seen in 4.6, both seeds stored in

FV (2507.2) and PV (2249.5) performed better than the seeds stored in ATS packaging (1523.5)

At 9 and 12 MAS, same trend was observed in which both factors, packaging types and storage temperature independently affecting the SVI with no interaction observed. Based on packaging types, seeds that were stored in FV condition (1,950 at 9 MAS, 1,994.83 in 12 MAS) always performed the best followed by the seeds in PV (1,838.9 at 9 MAS, 1,537.21 at 12 MAS) and the lowest was observed in ATS packaging (1,406.7 at 9 MAS, 1,212.66 at 12 MAS) with all three was significantly differed to one another.

Table 4.6: Summary of seedling performance based on seedling vigour index of sweet corn stored in different packaging type and stored in two different storage temperature at three months intervals of a year storage.

Source of Variation	Seedling vigour index			
	Storage Period			
	3 Months	6 Months	9 Months	12 Months
Packaging Type	ns	**	**	**
Full Vacuum	2,643.0	2,507.2 a	1,950.0 a	1,994.83 a
Partial Vacuum	2,641.2	2,249.5 a	1,838.9 b	1,537.21 b
Air-tight Sealed	2,480.8	1,523.5 b	1,406.7 c	1,212.66 c
Storage Temperature	ns	Ns	**	**
16°C	2,668.6	2,193.4	2,472.8 A	2,535.36 A
26±3°C	2,508.1	1,993.3	990.9 B	627.77 B
Packaging Type × Storage Temperature	ns	ns	ns	ns

** indicates highly significant differences at P ≤ 0.0001.
* indicates a significant differences at P ≤ 0.05.
ns indicates no significant differences.
means with same letter vertically in each of source of variation row sections are not significantly different at P > 0.05 using Tukey Test.

4.3.7 Seedling Length

Like other parameters explained before, there was no influence of packaging types and storage temperature on seedling length up to 3 MAS as can be seen in Table 4.7 below.

Table 4.7: Summary of seedling performance based on seedling length of sweet corn stored in different packaging type and stored in two different storage temperature at three months intervals of a year storage.

Source of Variation	Seedling length			
	Storage Period			
	3 Months	6 Months	9 Months	12 Months
Packaging Type	ns	**	**	**
Full Vacuum	29.19	28.37 a	25.48 a	26.38 a
Partial Vacuum	29.81	27.92 a	24.92 a	24.14 b
Air-tight Sealed	29.69	22.04 b	20.81 b	19.81 c
Storage Temperature	ns	*	**	**
16°C	29.43	27.19 A	28.78 A	29.64 A
26±3°C	28.37	25.02 B	18.69 B	17.25 B
Packaging Type × Storage Temperature	ns	* (Refer Figure 4.8)	ns	** (Refer Figure 4.9)

** indicates highly significant differences at P ≤ 0.0001.
* indicates a significant differences at P ≤ 0.05.
ns indicates no significant differences.
means with same letter vertically in each of source of variation row sections are not significantly different at P > 0.05 using Tukey Test.

At 6 MAS, there was interaction was observed between both factors on the seedling length and illustrated in Figure 4.8. All seeds stored in FV and PV on both 16°C, and 26±3°C room temperature had same performance in terms of seedling length, in exception the seeds packed in ATS packaging and stored

in 26±3°C room temperature which had shortest seedling length around 19.37 cm /seedling.

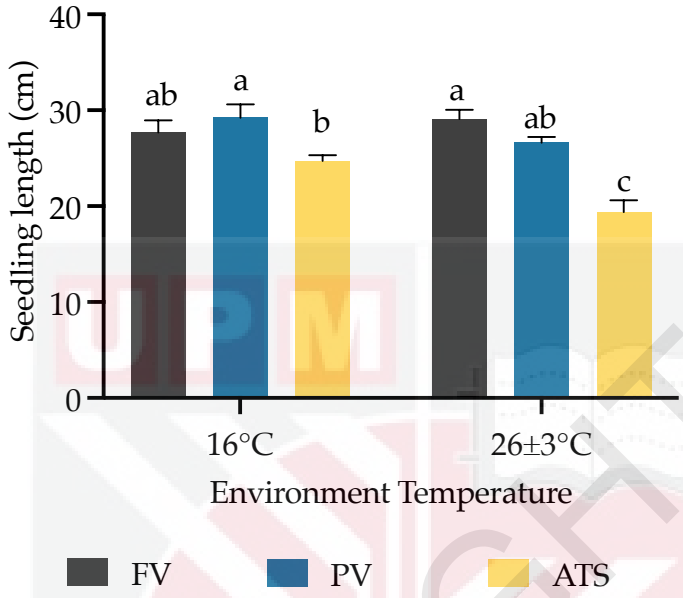

Figure 4.8: Seedling length as affected by both packaging types and storage temperature at 6 months. FV is full vacuum, PV is partial vacuum while ATS is air-tight sealed packaging. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

However, in the 9 MAS, there was no interaction was observed despite both factors independently influenced the seedling length (Refer Table 4.7). It was observed that the seeds stored in FV (25.48 cm /seedling) and PV (24.92 cm /seedling) would produce better seedling as measured based on its length compared to the seeds stored in ATS packaging which was only around 20.81 cm per seedling. Based on the storage temperature, seeds stored in 16°C would produce better seedling with length around 28.78 cm /seedling compared to seeds stored in 26±3°C room temperature which was only around 17.26 cm/seedling.

Interaction was observed again at 12 MAS and illustrated in Figure 4.9. All the seeds stored in 16°C had superior seedling length compared to 26±3°C room temperature. In 16°C, seedling produced by FV (30.91 cm /seedling) and PV (30.33 cm /seedling) seed had equal performance and better than ATS packaging (27.69 cm /seedling). All seed in 26±3°C room temperature had reduced length but all three was significantly differed one to another. Seed stored in FV produced around 21.86 cm per seedling and the shortest was observed in ATS packaging (11.93 cm /seedling).

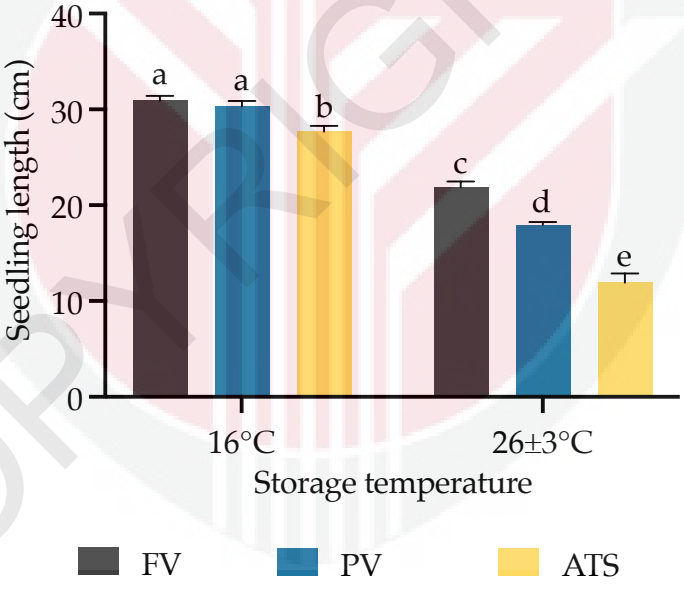

Figure 4.9: Seedling length as affected by both packaging types and storage temperature at 12 months. FV is full vacuum, PV is partial vacuum while ATS is air-tight sealed packaging. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

4.3.8 Seedling Shoot Dry Weight

Shoot dry weight was not affected when the seeds stored up to 3 MAS. However, packaging types was observed to affect shoot dry weight of sweet corn seedlings in Table 4.8. Seed in FV (0.034 g /seedling) and PV (0.033 g/ seedling) had an equal performance compared to ATS packaging (0.025 g/ seedling).

Table 4.8: Summary of seed vigour based on shoot dry weight of sweet corn stored in different packaging type and stored in two different storage temperature at three months intervals of a year storage.

Source of Variation	Shoot dry weight			
	Storage Period			
	3 Months	6 Months	9 Months	12 Months
Packaging Type	ns	**	**	**
Full Vacuum	0.031	0.034 a	0.028 a	0.028 a
Partial Vacuum	0.031	0.033 a	0.025 a	0.021 b
Air-tight Sealed	0.032	0.025 b	0.021 b	0.017 c
Storage Temperature	ns	ns	**	**
16°C	0.032	0.032	0.029 A	0.030 A
26±3°C	0.032	0.030	0.019 B	0.014 B
Packaging Type × Storage Temperature	ns	ns	ns	** (Refer Figure 4.10)

** indicates highly significant differences at P ≤ 0.0001.
* indicates a significant differences at P ≤ 0.05.
ns indicates no significant differences.
means with same letter vertically in each of source of variation row sections are not significantly different at P > 0.05 using Tukey Test.

There was no interaction was observed when the seeds stored up to 9 MAS but both factors independently affecting the shoot dry weight. As summarized in Table 4.8 earlier, seeds stored in FV (0.028 g /seedling) and PV (0.025 g /seedling) had equal performance while the poorest shoot dry weight obtained from the seeds stored in ATS packaging (0.021 g /seedling). Based in on storage temperature, seeds stored in 16°C (0.029 g /seedling) had always produce heavier shoot mass compared to 26±3°C room temperature (0.019 g /seedling).

At the end of storage period, there was interaction between packaging types and storage temperatures on the shoot dry weight in which seeds in FV and PV in 16°C (both at 0.033 g /seedling) had better performance compared to the rest. However, there was no significant difference between PV (0.030 g/ seedling) and ATS (0.028 g /seedling) packaging in the 16°C cold storage. Like most of other parameter, the seeds stored in 26±3°C room temperature would produce less quality of seedling as all seeds stored in this condition produced lighter shoot mass compared to 16°C cool storage with the lowest when seed stored in ATS packaging (0.006 g /seedling).

However, when compared based on packaging types, the seeds stored in FV performed better than PV and ATS packaging. All of this is summarized in Figure 4.10.

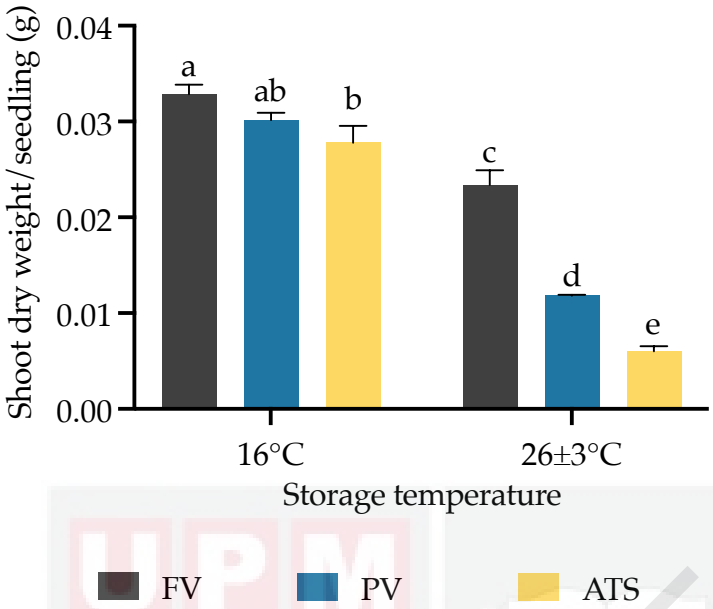

Figure 4.10: Shoot dry weight as affected by both packaging types and storage temperature at 12 months. FV is full vacuum, PV is partial vacuum while ATS is air-tight sealed packaging. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

4.3.9 Seedling Root Dry Weight

Root dry weight also was not affected by both factors studied when the seeds stored up to 3 MAS as can be seen in Table 4.9. However, there was interaction was observed in 6 MAS.

Table 4.9: Summary of seed viability based on root dry weight of sweet corn stored in different packaging type and stored in two different storage temperature at three months intervals of a year storage.

Source of Variation	Root dry weight			
	Storage Period			
	3 Months	6 Months	9 Months	12 Months
Packaging Type	ns	**	**	**
Full Vacuum	0.020	0.22 a	0.018 a	0.018 a
Partial Vacuum	0.020	0.021 a	0.015 b	0.014 b
Air-tight Sealed	0.019	0.018 b	0.012 c	0.010 c
Storage Temperature	ns	**	**	**
16°C	0.019	0.022 A	0.019 A	0.019 A
26±3°C	0.020	0.019 B	0.011 B	0.009 B
Packaging Type × Storage Temperature	ns	* (Refer Figure 4.11)	ns	* (Refer Figure 4.12)

** indicates highly significant differences at P ≤ 0.0001.
* indicates a significant differences at P ≤ 0.05.
ns indicates no significant differences.
means with same letter vertically in each of source of variation row sections are not significantly different at P > 0.05 using Tukey Test.

As can be seen in Figure 4.11, there was no significant different between seed stored 16°C (across all packaging types – between 0.022 to 0.023 g /seedling) with the seeds stored in vacuum condition (0.0213 g /seedling) that were placed in 26±3°C temperature. The lowest performance was observed in ATS packaging (0.0146 g /seedling).

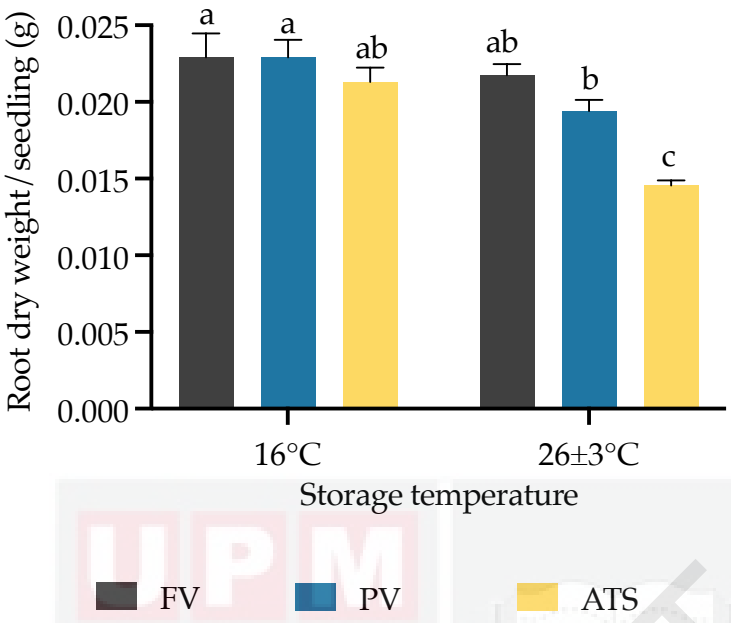

Figure 4.11: Root dry weight as affected by both packaging types and storage temperature at 6 months after storage. FV is full vacuum, PV is partial vacuum while ATS is air-tight sealed packaging. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

However, at 9 MAS, there was no interaction observed despite both factors influenced root dry weight of the seedlings. Based on packaging types, seeds stored in FV (0.018 g /seedling) would produce heavier root dry weight compared to PV (0.015 g /seedling) with the lowest was observed in ATS packaging (0.012 g /seedling). Better root dry weight can be obtained when the seed stored in 16°C cold room storage (0.019 g /seedling) compared to the seeds stored in 26±3°C temperature (0.011 g /seedling).

There was interaction was observed between packaging types and storage temperature as can be seen in Figure 4.12 at 12 MAS. Root dry weight was the best when seeds packed in FV (0.021 g /seedling) and PV (0.019 g /seedling) that were stored in 16°C. However, the seeds in PV performed

equally well with the seeds in ATS (0.016 g /seedling) making the seed in FV to be superior to the rest. Interestingly, the seeds packed in FV despite being stored in 26±3°C temperature had same performance with the seeds stored in air-tight packaging stored in 16°C. Seeds in air-tight packaging and stored in 26±3°C temperature would have reduced root dry weight (0.004 g /seedling) compared to other treatments.

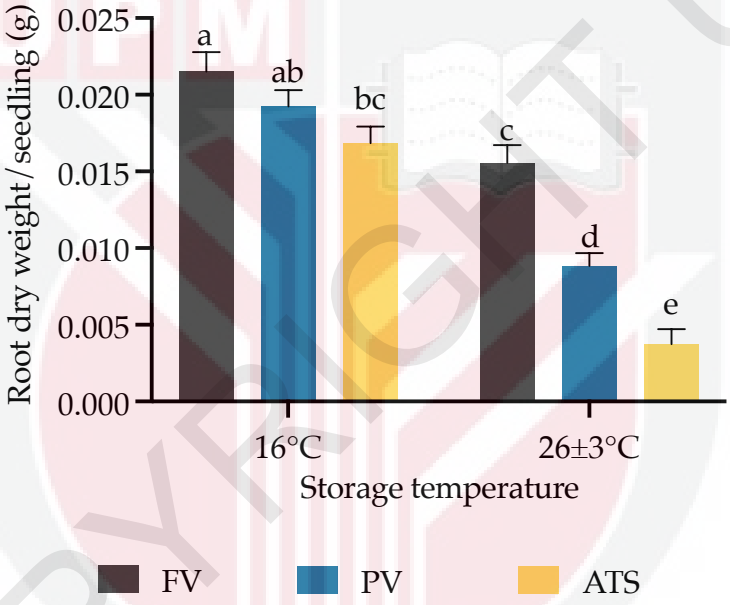

Figure 4.12: Root dry weight as affected by both packaging types and storage temperature at 12 months. FV is full vacuum, PV is partial vacuum while ATS is air-tight sealed packaging. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

4.3.10 Changes in Catalase Activity

Like other seed vigour and seedling performance parameters, the CAT enzyme activity was not influenced by both packaging types and storage temperature up to 3 MAS. Throughout 12 MAS, there was no interaction was

observed between both factors. However, in the 6 and 12 MAS, both factors independently affecting CAT enzyme activity in sweet corn seeds while only storage temperature affect the enzyme activity in the 9 MAS. Summary of finding can be seen in Table 4.10.

Table 4.10: Summary of antioxidant activity of catalase enzyme in sweet corn stored in different packaging type and stored in two different storage temperature at three months intervals of a year storage.

Catalase enzyme activities				
Source of Variation	**Storage Period**			
	3 Months	**6 Months**	**9 Months**	**12 Months**
Packaging Type	ns	**	ns	*
Full Vacuum	11.82	11.06 a	9.52	9.30 a
Partial Vacuum	11.82	11.17 a	9.39	8.889 ab
Air-tight Sealed	11.67	9.68 b	8.81	8.29 b
Storage Temperature	ns	*	**	**
16°C	11.97	11.12 A	10.21 A	9.63 A
26±3°C	11.57	10.09 B	8.27 B	8.02 B
Packaging Type × Storage Temperature	ns	ns	ns	ns

** indicates highly significant differences at P ≤ 0.0001.
* indicates a significant differences at P ≤ 0.05.
ns indicates no significant differences.
means with same letter vertically in each of source of variation row sections are not significantly different at P > 0.05 using Tukey Test.

During the 6 MAS in storage, seeds stored in FV (11.06 µmol /min /mg FW) and PV (11.17 µmol /min /mg FW) had equivalent CAT activity and higher compared to ATS packaging which was only around 9.68 µmol/min/mg FW. While there was no significant difference was observed during the 9 MAS, the same trend was observed at the end of 12 MAS where the highest CAT activity was observed in FV seeds which was around 9.30 µmol /min /mg FW. This

was followed by seeds stored in PV (8.89 µmol /min /mg FW) and ATS packaging (8.29 µmol /min /mg FW), in which there was no significant difference between the two packaging.

Based on storage temperature, the same trend between 6 to 12 MAS was observed in which seeds stored in 16°C cool room storage had higher CAT activity compared to the seeds stored in 26±3°C temperature. For example, the CAT activity was measured at 11.12 µmol /min /mg of fresh weight in 16°C compared to 26±3°C temperature that was around 10.08 µmol/min/mg FW. There was reduction in CAT activity in 9 MAS (10.21 µmol /min /mg FW in 16°C and 8.27 µmol /min /mg FW in 26±3°C room temperature) and 12 MAS (9.63 µmol /min /mg FW in 16°C and 8.02 µmol /min /mg FW in 26±3°C room temperature).

4.3.11 Changes in Guaiacol Peroxidase Activity

POD enzyme activity was affected by both packaging types and storage temperature as early as 3 MAS to 6 MAS although there was no interaction was observed between both factors (Refer Table 4.11).

In the 3 MAS, seed packed in FV (17.47 nmol /min /mg FW) and PV (15.02 nmol /min /mg FW) had equivalent POD activity but there was no significant difference between PV and ATS packaging (12.12 nmol /min /mg FW) making POD activity in FV was better. Interesting to observed that POD

activity increased in the 6 MAS for all packaging types where the seeds in FV and air-tight packaging had the highest activity. Surprisingly, the highest POD activity was observed in ATS packaging (22.99 nmol /min /mg FW) although it had not significantly differed with FV seed (22.02 nmol /min /mg FW) with the lowest POD activity was observed in PV seed (18.79 nmol /min /mg FW). POD activity was further increased in 9 MAS ranging between 32.54-33.68 nmol /min /mg FW with no significant difference was observed based on the packaging types.

Based on storage temperature, a consistent trend was observed between 3 to 6 MAS, with seeds stored at 16°C showing higher POD activity compared to those stored at 26±3°C. Over this period, POD activity gradually increased, peaking at 9 MAS. At 3 MAS, the POD activity of seeds stored at 16°C was 19.97 nmol /min /mg FW, which was 105% higher than the activity in seeds stored at 26±3°C (9.74 nmol /min /mg FW). By 6 MAS, the POD activity in seeds stored at 16°C increased to 25.25 nmol /min /mg FW, while seeds stored at 26±3°C showed a smaller increase to 17.28 nmol /min /mg FW, a difference of 46%. At 9 MAS, the highest POD activity was recorded in seeds stored at 16°C, with a value of 38.00 nmol /min /mg FW, which was 33% higher than that of seeds stored at 26±3°C (28.56 nmol /min /mg FW).

Table 4.11: Summary of antioxidant activity of guaiacol peroxidase enzyme in sweet corn stored in different packaging type and stored in two different temperature at three months intervals of a year storage.

Guaiacol peroxidase enzyme activities				
Source of Variation	**Storage Period**			
	3 Months	**6 Months**	**9 Months**	**12 Months**
Packaging Type	*	*	ns	**
Full Vacuum	17.47 a	22.02 ab	33.62	22.17
Partial Vacuum	15.02 ab	18.79 b	33.68	16.19
Air-tight Sealed	12.12 b	22.99 a	32.54	14.09
Storage Temperature	**	**	**	**
16°C	19.97 A	25.25 A	38.00 A	21.49 A
26±3°C	9.74 B	17.28 B	28.56 B	13.49 B
Packaging Type × Storage Temperature	ns	ns	ns	* (Refer Figure 4.13)

** indicates highly significant differences at P ≤ 0.0001.
* indicates a significant differences at P ≤ 0.05.
ns indicates no significant differences.
means with same letter vertically in each of source of variation row sections are not significantly different at P > 0.05 using Tukey Test.

At the end of 12 MAS, there was interaction between packaging types and storage temperature as illustrated in Figure 4.13. There was no difference in all packaging types in 16°C. Interestingly, the seeds packed in FV that were stored in 26±3°C room temperature had same POD activity as in 16°C storage. However, the seeds packed in PV and ATS packaging had poor and low activity of POD as expected.

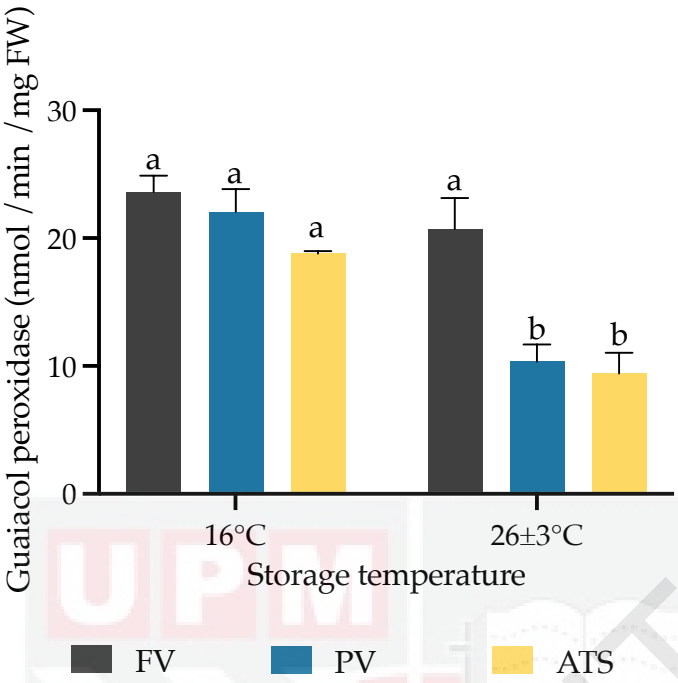

Figure 4.13: Guaiacol peroxidase content of seed after being stored for 12 months under different storage temperature and packaging types. FV is full vacuum, PV is partial vacuum while ATS is air-tight sealed packaging. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

4.3.12 Changes in Superoxide Dismutase Activity

Based on ANOVA, there was no significant effect of different packaging types and storage temperature observed at 3 MAS. However, between 6 to 12 MAS, there was independent effects of both factors on the SOD activity within the seeds. SOD activity based on the packaging types is illustrated in Figure 4.14. There was no difference in 3 MAS and its SOD content was ranging between 4.21-4.63 unit /mg /FW. During the 6 MAS, SOD was higher in seeds stored in ATS packaging (5.21 unit /mg /FW) and was followed by seeds in PV package (4.65 unit /mg /FW). The lowest activity was observed in the seeds stored in FV condition (4.42 unit /mg /FW).

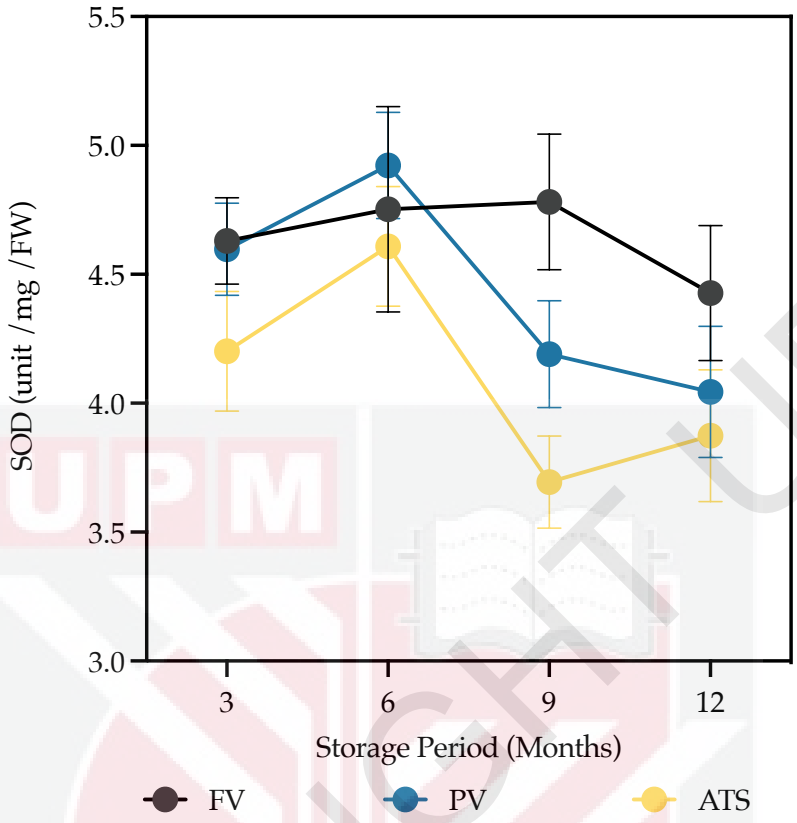

Figure 4.14: Superoxide dismutase activity changes when stored at three different packaging types for 12 months. FV is full vacuum, PV is partial vacuum while ATS is air-tight sealed packaging. Overlapped bars are not significantly different at P > 0.05 using Tukey Test.

At 9 MAS, a reduction in SOD activity was observed compared to 6 MAS. In contrast to 6 MAS, the highest SOD activity was recorded in FV packaging (4.78 unit /mg /FW), which was 13.4% higher than the activity in PV packaging (4.19 uni t/mg /FW). The lowest SOD activity was observed in ATS packaging (3.69 unit /mg /FW), which was 12.0% lower than PV and 22.8% lower than FV. All three packaging types showed significant differences in SOD activity at 9 MAS.

At 12 MAS, SOD activity remained high in FV packaging (4.43 unit/mg/FW), but no significant difference was observed between FV and PV packaging (4.04 unit/mg/FW). However, seeds stored in PV packaging showed no significant difference in SOD activity compared to those stored in ATS packaging (3.87 unit/mg/FW), with PV and ATS showing a 5.0% and 4.2% difference, respectively

At 3 MAS, no significant difference in SOD activity was observed between seeds stored at 16°C and those stored at 26±3°C. However, between 6 to 12 MAS, seeds stored at 16°C consistently exhibited higher SOD activity compared to those stored at 26±3°C room temperature. At 6 MAS, the SOD activity in seeds stored at 16°C was 5.09 unit /mg /FW, which was 15% higher than that in seeds stored at 26±3°C (4.43 unit /mg /FW).

Over time, the SOD activity in seeds stored at 26±3°C progressively decreased, while seeds stored at 16°C showed a slight decrease between 6 and 9 MAS but remained stable from 9 to 12 MAS. For example, at 9 MAS, SOD activity in seeds stored at 16°C was 4.66 unit /mg /FW, and at 12 MAS, it remained steady at 4.70 unit /mg /FW. In contrast, the SOD activity in seeds stored at 26±3°C continued to decline, dropping to 3.79 unit /mg /FW at 9 MAS and further decreasing to 3.53 unit /mg /FW by the end of the storage period, representing a 20% decrease from the initial measurement at 6 MAS.

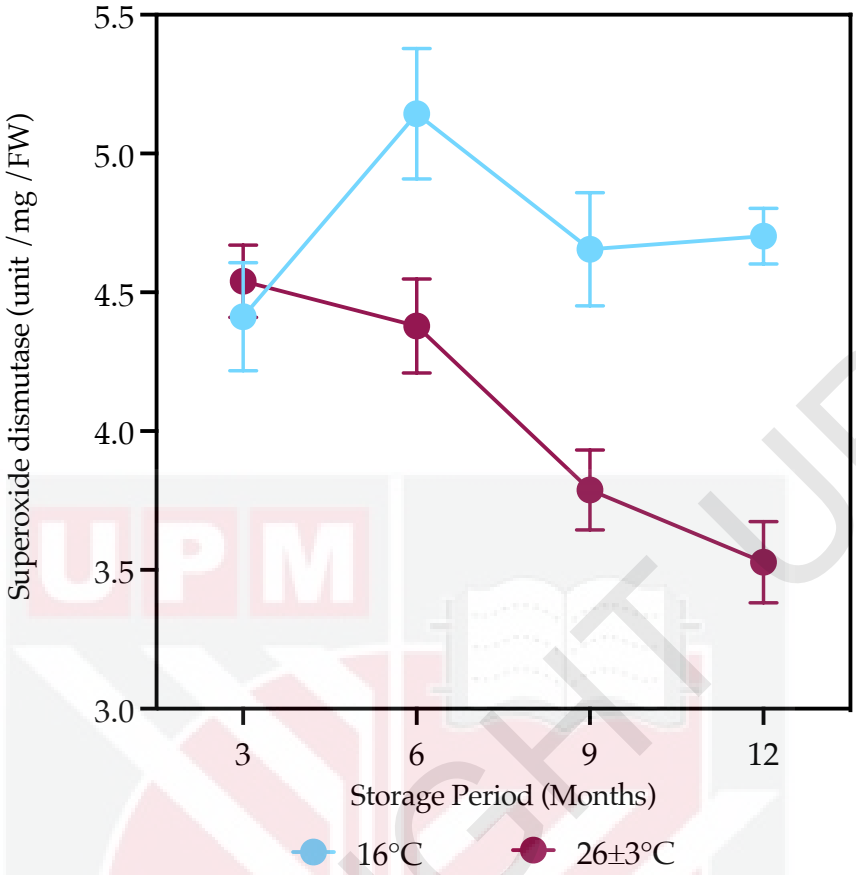

Figure 4.15: Superoxide dismutase activity changes when stored at two different storage temperature for 12 months. Overlapped bars are not significantly different at P > 0.05 using Tukey Test.

4.3.13 Changes in Malondialdehyde Content

Throughout 12 MAS, there was no interaction found, but the factors independently affecting the MDA content throughout the storage as can be seen in Table 4.12. At 3 MAS in storage, packaging types was observed to influence the lipid peroxidation as measured by MDA activity. High MDA content was observed in ATS packaging which was around 2.13 mmol /g FW. Lesser MDA content was found on the other two packaging types, 1.77 mmol /g FW in PV and the lowest in FV (1.54 mmol /g FW). However, there was no significant difference was found when the seeds stored for the next few MAS. The

difference was only observed at the end of storage study. MDA content was found to increase in all packaging types. However lesser lipid disruption was found in FV (3.27 mmol /g FW) compared to ATS packaging that was around 3.96 mmol /g FW.

Table 4.12: Summary of lipid peroxidation activity measured by malondialdehyde content in sweet corn stored in different packaging type and stored in two different storage temperature at three months intervals of a year storage.

Source of Variation	Malondialdehyde content			
	Storage Period			
	3 Months	6 Months	9 Months	12 Months
Packaging Type	**	ns	ns	**
Full Vacuum	1.54 b	2.63	3.07	3.27 b
Partial Vacuum	1.77 b	2.77	3.30	3.60 ab
Air-tight Sealed	2.13 a	2.94	3.32	3.96 a
Storage Temperature	ns	**	*	**
16°C		(Refer Figure 4.16)		
26±3°C				
Packaging Type × Storage Temperature	ns	ns	ns	ns

** indicates highly significant differences at P ≤ 0.0001.
* indicates a significant differences at P ≤ 0.05.
ns indicates no significant differences.
means with same letter vertically in each of source of variation row sections are not significantly different at P > 0.05 using Tukey Test.

As illustrated in Figure 4.16, there was no significant effect of storage temperature on MDA content at 3 MAS. However, from 6 MAS onwards, temperature had a clear effect on MDA accumulation, with seeds stored at 26±3°C consistently showing higher MDA levels compared to those stored at 16°C. At 3 MAS, the MDA content was very similar for both storage conditions,

at 1.81 mmol /g FW for seeds stored at 16°C and 1.82 mmol /g FW for seeds stored at 26±3°C. At 6 MAS, the MDA content in seeds stored at 16°C increased to 2.36 mmol /g FW, while seeds stored at 26±3°C showed a significantly higher MDA content of 3.21 mmol /g FW, representing a 36% increase in MDA in seeds stored at 26±3°C compared to those at 16°C. Although MDA content continued to increase in seeds stored at 16°C, it remained lower throughout the storage period, ranging from 2.85 to 2.94 mmol /g FW. In contrast, MDA content in seeds stored at 26±3°C continued to rise, reaching 3.60 mmol /g FW at 9 MAS and peaking at 4.29 mmol /g FW at the end of the observation period, reflecting an overall increase of 33% from 6 MAS to the end of storage.

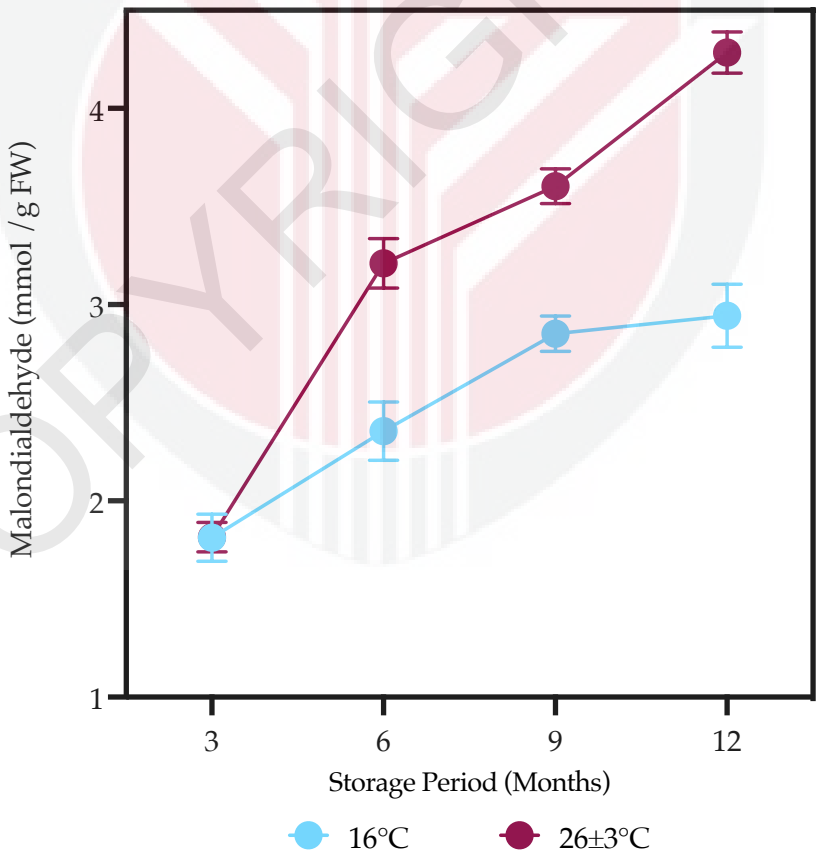

Figure 4.16: Malondialdehyde changes when stored at two different storage temperature for 12 months. Overlapped bars are not significantly different at P > 0.05 using Tukey Test.

4.4 Discussions

Seed Viability and Vigour During Storage

Seed viability is the most important parameter in determining seed quality. Up to 3 MAS in this study no significant changes in seed viability of stored sweet corn seeds were observed. In rice study by Wang et al. (2018), there was no different observed in seed viability when the seed stored in room temperature to the seed stored in cool temperature, provided the seed stored in vacuum packaging up, until the end of 60 days period. In comparison to this study, no significant difference was observed up until 3 MAS, despite different packaging used. However, an effect was observed in packaging types after 6 MAS in storage (approximately 180 days). Seed viability was initially at 97% but reduced to 88.5 in FV to 68.5% in ATS packaging. Interestingly, up to 180 days, there was no significant difference in seed viability of seeds stored in 26±3°C room temperature with the seed stored in 16°C environment, ranging between 78 to 80.3%. While storing in 16°C is more effective, with proper packaging, seed ageing can be slowed down up to 180 days in this study. In some cases, seed deterioration can be temperature dependent such as in Brassica plants (Mira et al., 2015) but Wang et al. (2018) suggested seed moisture has greater role in slowing down seed ageing and vacuum condition maintained the state of low moisture as there is no exchange of outside moisture and water.

In comparison to study by De Camargo & De Carvalho, (2008), where storing sweet corn seed in FV condition managed to slow down seed ageing up to 18 MAS, in this study however, difference was observed after 9 MAS where seed stored in cool room temperature maintain better viability compared to 26±3°C room temperature. Although in 6 MAS, result looks promising, but 12 MAS, storage at 16°C was still superior compared to 26±3°C room temperature. Storing in cool room (16°C) had the maize seeds viability than in 26±3°C room temperature based on Deuner et al. (2014).

However, seed viability can be further maintained when the seed stored in FV or PV. Seed viability maintained high (78-92%) in cool room storage with both vacuum and PV had the best FGP (>86%). Storage in 26±3°C temperature severely affect seed viability with the lowest viability, 22% in ATS packaging. This result had same trend with other studies such as in sunflower (Abreu et al., 2013), rye (Barzali et al., 2005), groundnut (Sastry et al., 2007) and chilli (Deepa et al., 2013). Other than FV, PV condition was also found to be effective to prolong life shell of primed bitter gourd seeds for 12 MAS (Yeh et al., 2005) and primed sweet corn for 18 months (Chiu et al., 2003). Viability of seed in relations to storage temperature and vacuum condition, can be compared with a study on mung bean, where storage in vacuum condition prolong seed viability more than 90% for 14 months in 20°C but only 6 months in 26±3°C temperature (Faisal et al., 2019).

Vacuum condition enables seeds to maintain its lower moisture due to inhibition of exchange of oxygen and water molecule reducing seed respiration (Deepa et al., 2013). This was seen in *Platycodon grandiflorum* seeds where there no changes in seed moisture content after 10 MAS (Feng et al., 2017). In cotton, moisture content increased from 8.3% to 12% within 18 months while in vacuum condition, it was stagnant at 8.23% and stark difference was observed in viability, 57% in nonvacuum and 80% in vacuum, indicating there is correlation between these two component. Apart from vacuuming itself, Metalized PE packaging also had better protection inhibiting exchange of moisture in rice (Alahakoon et al., 2021).

Seedling Performance During Storage

Seedling performance in terms of seedling length, root dry weight as well as shoot dry weight was improved when the seed stored in FV and PV in this study. Same trend observed in rye (Barzali et al., 2005) with vacuum packed seeds gave the highest seedling values such shoot dry weight, seedling dry weight, shoot length, root length, and seedling length. In paddy (SaidaNaik, 2018), seedling dry weight in vacuum bag is higher than ATS polyethylene bag and cloth bag, with there was no difference based on storing temperature (25°C and 4°C), however, this was not observed in this study. Seedling length, root dry weight and shoot dry weight was significantly differed between storage temperature environment despite both stored in vacuum condition.

This probably resulting from vacuum-delayed deterioration of antioxidative systems.

Seed Biochemical Activities During Storage

This finding suggested that in early stage of seed deterioration, seed ageing is more triggered by moisture content of the seed, but in long run, temperature is important in slowing down the metabolic activities within the seed. Storing in ATS packaging provides a hermetic condition but with air contained within the packaging, compared to FV where air is exhausted from the packaging, leaving seeds with a very minimal amount of air (Chandra et al., 2022). Storage under aerobic condition had higher rate of respiration compared to anaerobic in FV and, triggering many physiological activities related to respiration such as depletion of seed reserve, oxidation of lipid and many others(Ellis et al., 1991). It was observed that seed stored in low moisture condition had better stability of antioxidant mechanism especially antioxidant enzymes and this can be observed in *Calocedrus macrolepis* (Zhang et al., 2019)

Depletion of protective antioxidant mechanism of can be associated with proliferation of oxygen level in packaging (Wilson & McDonald, 1986). In this study, three antioxidant defence enzymes, CAT, POD, and SOD were measured and throughout 12 MAS, it declines as the ageing progressing. In Chiu et al. (2003), the same trend was observed where SOD, CAT and APX is declining within 18 MAS, but the rate was slower in PV than non-vacuum.

A study on oat seeds showed that enzymatic antioxidants such as CAT, POD, and SOD can protect against oxidative stress in stored seeds with low moisture content (Kong et al., 2015). Seed ageing might be reduced if there is lesser oxidative stress in oxygen free storage atmosphere and marked by changes in content and activity of antioxidant enzymes (Barzali et al., 2005). Study by Chiu et al. (2003) showed higher antioxidant enzymes were maintained in vacuum packaging compared to non-vacuum. In this study, SOD content was higher in vacuum stored seeds compared to ATS seeds, and in line with the seed viability and vigour parameters. This supports the argument that SOD helps in defending ROS damage by neutralizing super oxide, hence provide protection to the seed(Wilson & McDonald, 1986). Yeh et al. (2005) stated that there was a positive correlation between seed germination percentage with SOD content backing up the roles of antioxidant enzymes in maintaining seed quality. In this study, it was also observed in CAT and POD content in FV than ATS was higher at the end of 12 MAS where CAT roles are to decompose H_2O_2 to water and oxygen molecule while POD neutralized hydroxyl (Zhang et al., 2021).

The reduction in antioxidant to counter back oxidative damage, such as CAT, POD and SOD may result in higher lipid peroxidation as ROS starts to accumulate and it is responsible for alterations of membrane permeability because of the changing compositions of fatty acids and phospholipids (Kalemba et al., 2015; Wojtyla et al., 2016). The seed ageing of sweet corn seed

can be measured by the amount of MDA as the seed progressed deterioration within 12 MAS. Lipid peroxidation is much higher in deteriorated seed as reflected in MDA content (Ayala et al., 2014). Both of the factors independently affecting seed's MDA content. Highest MDA was observed in ATS packaging (3.97 mmol/g) and the lowest in FV (3.27mmol/g). Seeds stored in 26±3°C temperature had higher average of 4.29 mmol/g compared to seeds at 16°C cool room (2.94 mmol/g). This indicates that seeds stored in FV is managed to reduce the lipid peroxidation within the seeds while cool room temperature also had the same effect. In other study, amount of MDA and seed leachates in peanut placed woven packaging was twice than the seeds stored between -0.06 to -0.09mPa vacuum PE bag Wang et al. (2016). According to Johnson & Decker (2015), oxygen amount needed to cause oxidative damage is very small, and anaerobic condition can to 2% can reduce or limit the lipid oxidation, hence why the vacuum condition able to mitigate the damage on the lipid layer of cell.

4.5 Conclusion

Environment during seed storage is very important in maintaining seed quality and to prolong its shelf-life. Understanding the factors that causes seed ageing can help us to find the best way to storage seeds, reduce deterioration and increase longevity for sweet corn which is known to have storability issue.

Based on this study, seeds should be stored in FV condition (-0.09 to -0.1 mPa) to prolong seed viability and quality. GSH11005Y sweet corn seed viability was maintained above 80% even after 12 MAS when stored in this condition. For long term seed storage, seeds should be stored in cool room (16°C), but for short term storage (less than 6 months), it is sufficient to store in 26±3°C temperature if stored in FV condition. Storing seeds in this vacuum condition and cool room storage, slows down the rate of deterioration, reduces depletion of antioxidant mechanism, maintains seed viability and improvs its vigour.

This can be observed in lower MGT, higher GI, faster seed germination based on coefficient velocity of germination, higher seedling dry mass and length and antioxidant enzymes such as POD, CAT and SOD can be maintained high, which help to prolong sweet corn seed shelf-life.

CHAPTER 5

HYDOGEN PEROXIDE AS POTENTIAL PRIMING AGENT TO REINVIGORATE AGED SWEET CORN SEED

5.1 Introduction

Seed deterioration is an inevitable process and planting deteriorated seeds will lead to poor germination and stand establishment(Weerasekara et al., 2021). Seed ageing, caused by increment in moisture content, accumulation of ROS, and many environmental factors, reflected in many of morphological changes including increase in seed leachates, reduced respiration and loss on enzyme activities (Zhang et al., 2021).

Other than slowing down seed ageing through a good storage practice, pre-treatment such as priming could be beneficial to improve seed condition prior to sowing. Priming is a technique of controlling hydration and drying which results in more rapid germination and increase of seed vigour when re-imbibed (Bradford, 1986; Raj & Raj, 2019). Priming works in many ways, such as activation or elevation of certain enzymes or protein responding to germination, activation of cell repairing mechanism or also antioxidant defence mechanism (Gammoudi et al., 2020). Priming also is a cost-effective approach to reinvigorate seed to improve seedling establishment thus used everywhere around the world (Farooq et al., 2019). Osmo-priming is a

technique of soaking seed at different external water potential or in osmotic solution before rehydrating it back before re-imbibing seed for germination (Chen & Arora, 2011). Among the chemical that is used as osmo-priming agents are polyethylene glycol (PEG), mannitol, and potassium nitrate (Aliloo et al., 2008). Priming on sweet corn seed had been done from the simplest form of hydro-priming to nano priming as reviewed in Section 2.4.2. However, the chemical itself can be difficult to obtain especially to small farmers. Hydro-priming can be the cheapest option but most studied reviewed showed that it has the least improvement in seed vigour. Hence, identifying cheaper compound is very important to make sure it is accessible especially to small farmers.

Today, the use of H_2O_2 as pre-treatment to improve plant growth is gaining some attention. Lariguet et al. (2013) stated that H_2O_2 at proper concentration would help in breaking seed dormancy and improve germination while over accumulation would lead to cell injury which can be detrimental to the seeds (Kumar et al., 2010). Many studies have reported that application of H_2O_2 or combination of it with other compound as seed or seedling pe-treatment is useful as it induces and activates plant abiotic stress protective mechanism such as accumulation of latent defence mechanism, by maintaining non-toxic levels just enough to signal stress ROS scavenging actions (Hossain et al., 2015). Many antioxidant mechanism by plants such as SOD, CAT and POD have been attributed to redox balance from the act of signalling by H_2O_2 in plant (Ślesak et al., 2007).

H_2O_2 has been used as pre-treatment, such as foliar spraying and priming in many plants and in varied abiotic stress condition such as salinity, drought, different temperature regime as well as heavy metals (Banerjee & Roychoudhury, 2019). Among the crops, wheat (Hameed & Iqbal, 2014), rice (Jira-Anunkul & Pattanagul, 2020), and sunflower (Silva et al., 2020) showed that various application concentration (ranging between 1 µM to 100 mM) of H_2O_2 as priming were beneficial to seed viability and vigour as well as seedling performance, however, the effect of H_2O_2 on deteriorated sweet corn seeds has never been tested.

Therefore, this study was conducted with the aim to study the potential of H_2O_2 as priming agent to reinvigorate stored sweet corn seeds. The effect of priming using H_2O_2 at different concentration on sweet corn seed viability and vigour, seedling performance, and antioxidant activity particularly seed antioxidant enzymes were studied.

5.2 Materials and Methods

5.2.1 Location of Study

All laboratory work was conducted at the Seed Technology Laboratory, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor.

5.2.2 Seed Selection

A year-old GSH11005Y sweet corn seed collected at 35 DAP and stored in FV in 26±3°C room condition from Chapter 3 of this thesis was selected. Seed germination upon retrieval under the afore-mentioned condition was 50%.

5.2.3 Seed Priming

Seed initial moisture content was determined using the method described in Section 3.2.4 in Chapter 3.

Then, a preliminary study was conducted by soaking seeds in different priming concentrations ranging between 10 to 100 mM of H_2O_2 for 24 hours in room temperature based on the literature reviewed in Section 2.4.2. The objective is to screen appropriate range for priming concentration for sweet corn seed. After 24 hours, seeds were removed from the priming solution, and dried under room temperature between 3 to 4 days until it reached the initial

MC. Once the seeds reached the initial MC, seeds were subjected to germination test within 24 hours as explained in Section 5.2.4 with untreated seed as control.

After germination test result from preliminary study was obtained, another set of priming concentration was tested, that was between 1, 2.5, 5, 7.5, 10, 12.5, 17.5, and 20 mM of H_2O_2. Same procedure was used, where seeds were primed for 24 hours before redried back to initial MC. Seed germination was being conducted within 24 hours after achieving initial MC.

5.2.4 Seed Germination Test and Measurement of Seed Vigour

Seed germination test was conducted using the sand method (ISTA, 2017). Procedures of seed germination test was conducted as described in Section 3.2.8 in Chapter 3.

5.2.4.1 Final Germination Percentage

FGP was determined based on Section 3.2.8.1 in Chapter 3.

5.2.4.2 Mean Germination Time

MGT was determined based on Section 3.2.8.2 in Chapter 3.

5.2.4.3 Germination Rate Index

GRI was determined based on Section 3.2.8.3 in Chapter 3.

5.2.4.4 Germination Index

GI was determined based on Section 3.2.8.4 in Chapter 3.

5.2.4.5 Coefficient Velocity of Germination

CVG was determined based on Section 3.2.8.5 in Chapter 3.

5.2.4.6 Seedling Vigour Index

SVI was determined using method in Section 3.2.8.6 in Chapter 3.

5.2.4.7 Measurement of Seedling Length

Measurement of seedling length was determined based on Section 3.2.8.7 in Chapter 3.

5.2.4.8 Measurement of Shoot and Root Dry Weight

Measurement of Shoot and Root Dry Weight was determined using method in Section 3.2.8.8 in Chapter 3.

5.2.5 Electrical Conductivity of Seed Leachate

Electrical conductivity of seed leachate was determined using method explained in Section 3.2.9 in Chapter 3.

5.2.6 Determination and Assay of Antioxidant Enzymes

Preparation of seed sample and enzyme extracts was prepared using method described in Section 3.2.11 in Chapter 3.

5.2.6.1 Measurement of Catalase (EC 1.11.1.6)

Measurement and assay of CAT was conducted based on the protocol described in Section 3.2.11.1 in Chapter 3.

5.2.6.2 Measurement of Guaiacol Peroxidase (EC 1.11.1.7)

Measurement and assay of POD was conducted based on the protocol described in Section 3.2.11.2 in Chapter 3.

5.2.6.3 Measurement of Superoxide Dismutase (EC .1.15.1.1)

Measurement and assay of SOD was conducted based on the protocol described in Section 4.2.3.3 in Chapter 4.

5.2.7 Determination and Assay of Malondialdehyde

Measurement and assay of MDA was conducted based on the protocol described in Section 3.2.12 in Chapter 3.

5.2.8 Experimental Design and Data Analysis

The experiment was conducted using a complete randomized block design (RCBD) with different priming concentrations of H_2O_2 as the treatments, replicated four times. Each replicate was performed separately at different time and was treated as the blocks. ANOVA was carried out for all parameters and if significant difference was found, means comparison was done using Tukey Test with minimum 95% confidence interval. All statistical analysis were carried out using statistical software, Statistical Analysis System (SAS 9.4).

5.3 Results

5.3.1 Preliminary Study to Higher Hydrogen Peroxide Concentration up to 100 mM as Priming

Based on the H_2O_2 priming concentration up to 100 mM, there was a significant effect of it on seed viability (Refer Appendix 5.1). Means comparison is illustrated in Figure 5.1.

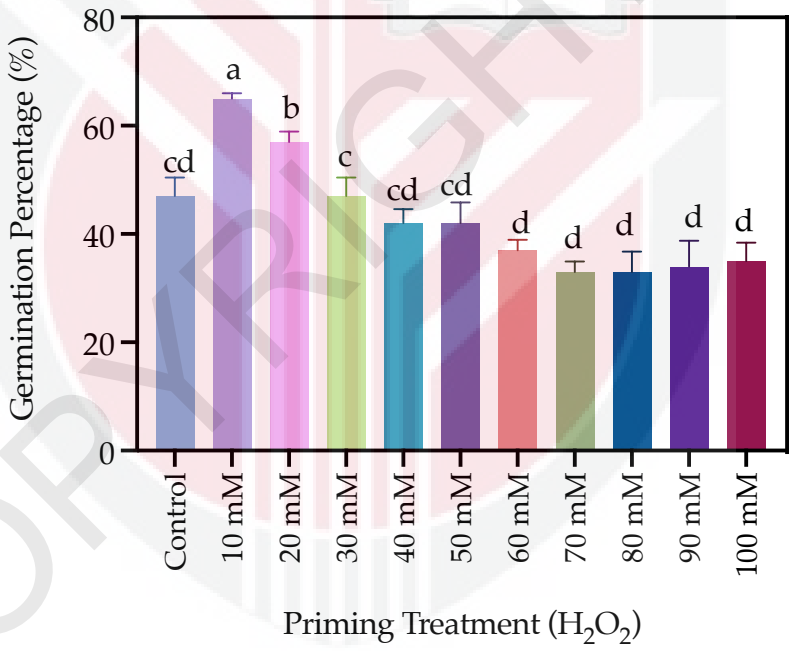

Figure 5.1: Final germination percentage of sweet corn seed primed between 0 to 100 mM of hydrogen peroxide for 24 hours. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

Untreated seed had germination percentage around 47% and priming at 10 mM provides the highest viability at 65% and 20 mM resulted in 57% germination. However, further priming more than 30 mM of H_2O_2 caused

further reduction in sweet corn seed viability ranging between 33 to 47% depending on concentration. Based on this finding, selected priming concentration ranging between 0 to 20 mM of H_2O_2 was repeated and the result is explained in the following subsection.

5.3.2 Hydrogen Peroxide Priming at 0 to 20 mM

Seed quality parameters in seed viability, seed vigour, seedling performance and seed biochemical activity such as antioxidant enzymes and lipid peroxidation were measured, and the finding presented as follow:

5.3.2.1 Final Germination Percentage

A significant difference was observed in the FGP when seeds were treated with different H_2O_2 concentration (Refer Appendix 5.2). Figure 5.2 shows that, compared to untreated seeds, all priming treatments did not result in a decrease in seed viability. However, seeds treated with H_2O_2 concentrations ranging from 5 mM to 10 mM exhibited significantly better germination rates than untreated seeds. Untreated seeds had the lowest viability at 48%. While seeds treated with H_2O_2 concentrations between 1 mM to 2.5 mM and 12.5 mM to 20 mM showed improved viability, with germination rates between 55% and 62%, Tukey's Test revealed no significant differences between these treatments and the untreated seeds. The only significant improvements in germination were observed with 5 mM, 7.5 mM, and 10 mM H_2O_2 treatments.

The highest germination (69%) was seen in seeds treated with 10 mM H_2O_2 for 24 hours, followed by seeds treated with 5 mM (67%) and 7.5 mM (66%) H_2O_2, with no significant difference between these three concentrations.

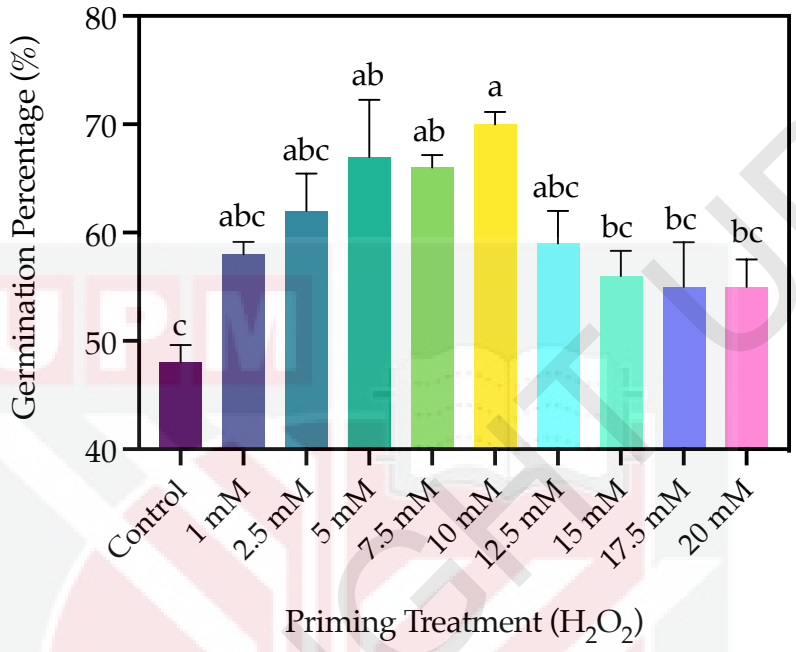

Figure 5.2: Final germination percentage of seeds treated with different concentration of hydrogen peroxide priming compared to untreated seeds. Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

5.3.2.2 Mean Germination Time

There was a significant difference observed in MGT when seeds were treated with different priming concentration (Refer Appendix 5.3). Post hoc means comparison was conducted and illustrated in Figure 5.3.

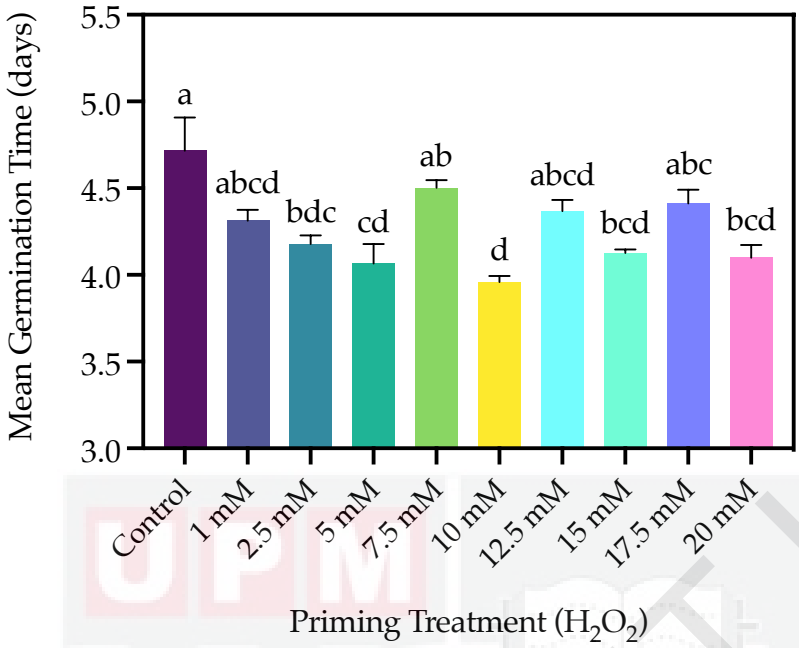

Figure 5.3: Mean germination time of seeds treated with different concentration of hydrogen peroxide compared to untreated seed (control). Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

The fastest germination time was observed when the seeds were primed in 10 mM of H_2O_2 for 24 hours which only took about 3.96 days to germination compared to untreated seed which took more than 4.72 days to germinate. Seeds primed at 2.5 mM, 5 mM, 15 mM and 20 mM of H_2O_2 also had better MGT ranging between 4.09 to 4.11 days and not significantly differed with 10 mM based on Tukey's Test. Seeds primed at 10 mM of H_2O_2 for 24 hours showed the highest performance, outperforming all other priming concentrations. Conversely, seeds primed at 1 mM, 7.5 mM, 12.5 mM, and 17.5 mM exhibited similar results to untreated seeds, with no marked improvement.

5.3.2.3 Germination Index

ANOVA results (Refer Appendix 5.4) showed that the GI of sweet corn seeds was significantly affected by different priming concentrations of H_2O_2 (Figure 5.4). All seeds treated with H_2O_2 exhibited an increase in GI, but only those primed with concentrations between 2.5 mM to 10 mM showed a statistically significant improvement compared to untreated seeds. The highest GI was observed in seeds treated with 10 mM H_2O_2 for 24 hours, achieving a GI of 291, which was nearly 84% higher than that of untreated seeds (158). Seeds primed at 5 mM showed a GI of 267, which was not significantly different from the 10 mM treatment, but still 69% higher than the untreated seeds.

Seeds treated at 7.5 mM and 2.5 mM had GIs of 231 and 237, respectively, which were significantly higher than the untreated seeds but lower than the 5 mM and 10 mM treatments. In contrast, seeds treated with 12.5 mM to 20 mM (except for 15 mM) showed higher GIs but were statistically indistinguishable from untreated seeds. The lowest GI was observed in untreated seeds (158).

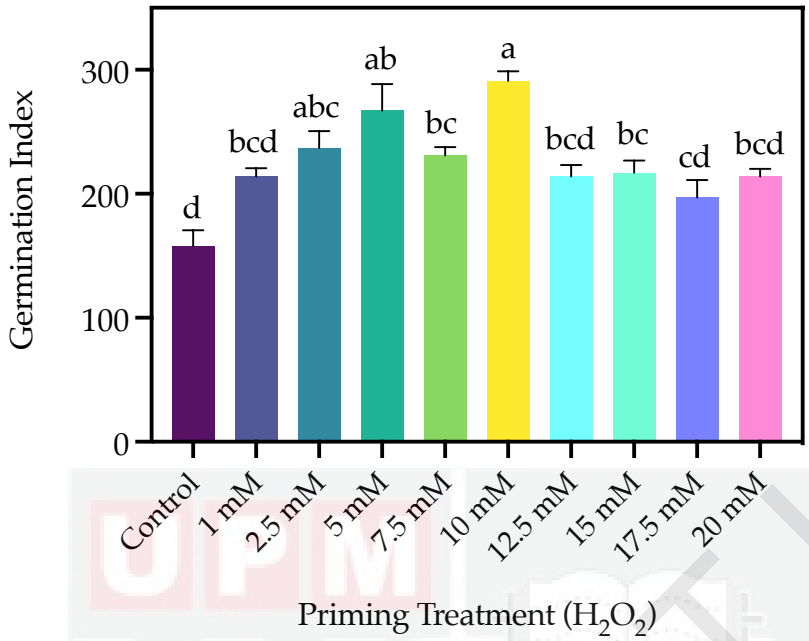

Figure 5.4: Germination index of seeds treated with different concentration of hydrogen peroxide compared to untreated seed (control). Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

5.3.2.4 Germination Rate Index

Different priming concentration of H_2O_2 between 0 to 20 mM of H_2O_2 influenced GRI of sweet corn seeds based on ANOVA in Appendix 5.5. Post hoc comparison of means is illustrated in Figure 5.5. The highest GRI was obtained when the seeds were primed in 10 mM H_2O_2 for 24 hours which was around 18.68 followed by priming at 5 mM (17.00) and 2.5 mM (15.13) which all significantly the same. However, the latter two was insignificant than other priming concentrations. Other priming concentration (1 mM, 12.5 mM to 20 mM) had no significant improvement in GRI (12.60 – 13.76) ranging between compared to untreated seeds which was only at 10.63.

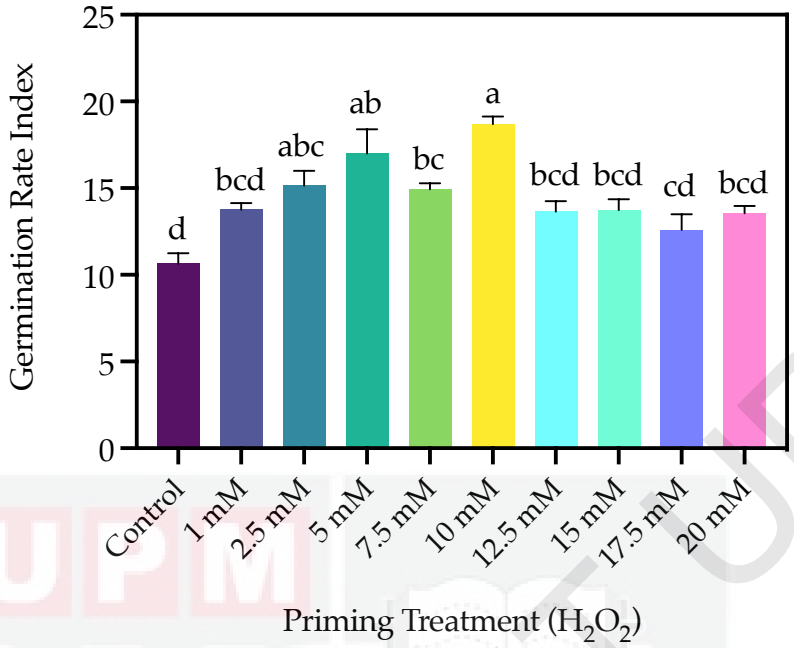

Figure 5.5: Germination rate index of seeds treated with different concentration of hydrogen peroxide compared to untreated seed (control). Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

5.3.2.5 Coefficient Velocity of Germination

Another seed vigour parameter, CVG of sweet corn seed was significantly affected by different priming H_2O_2 concentration between 0 to 20 mM (Refer Appendix 5.6). Figure 5.6 showed means comparison of CVG of sweet corn seeds when treated with different priming concentration. It showed that the highest CVG can be obtained when the seeds primed in 10 mM of H_2O_2 for 24 hours at 25.27 point. Seeds primed at 1 mM, 7.5 mM, 12.5 mM and 17.5 mM (22.22 – 23.21) had no significant difference with the control (untreated seeds) which was only at 21.31. Seeds treated with 2.5 mM, 5.5 mM, 15 mM and 20 mM of H_2O_2 although statistically equally well with 10 mM seeds, but it also

had equal performance with the seeds mentioned earlier (1 mM, 7.5 mM, 12.5 mM and 17.5 mM) making the seeds primed in 10 mM of H_2O_2 had superior CVG than other treatments tested in this study.

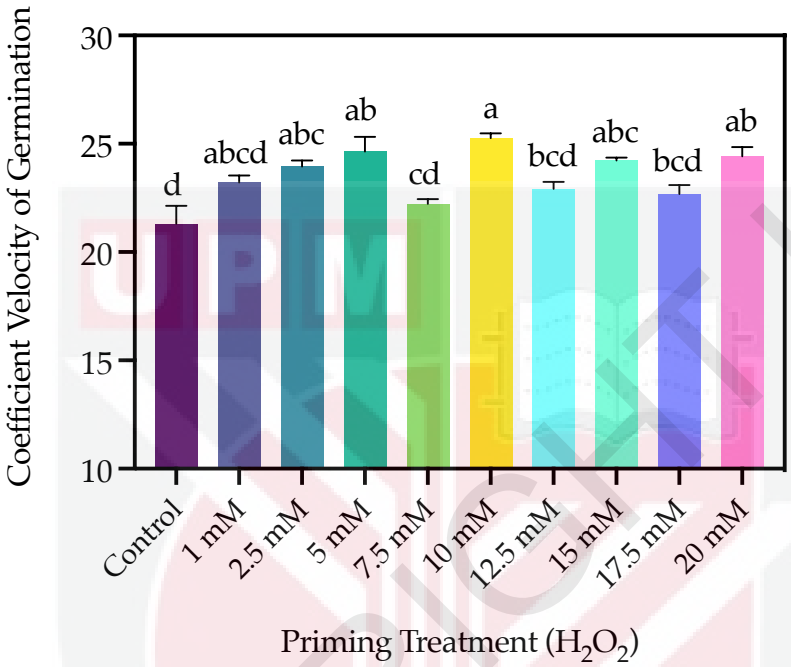

Figure 5.6: Coefficient velocity of germination of seeds treated with different concentration of hydrogen peroxide compared to untreated seed (control). Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

5.3.2.6 Seedling Length

Seedling performance parameters such as seedling length showed a significant difference when the sweet corn seeds were treated with different priming concentration of H_2O_2 for 24 hours (Refer Appendix 5.7). The longest seedling was obtained from seeds treated with 7.5 mM of H_2O_2 which was around 29.66 cm /seedling, followed by seeds primed at 10 mM H_2O_2 at 28.93 cm /seedling

and priming between 1 mM to 5 mM (27.63 – 28.72 cm /seedling) All treatments mentioned had no significant difference between them. However, priming between 1 mM to 5 mM perform equally same with 12.5 mM to 20 mM (25.28 – 25.93 cm /seedling) where it statically not differed with untreated seeds at 23.23 cm /seedling. For better understanding, Figure 5.7 is referred below.

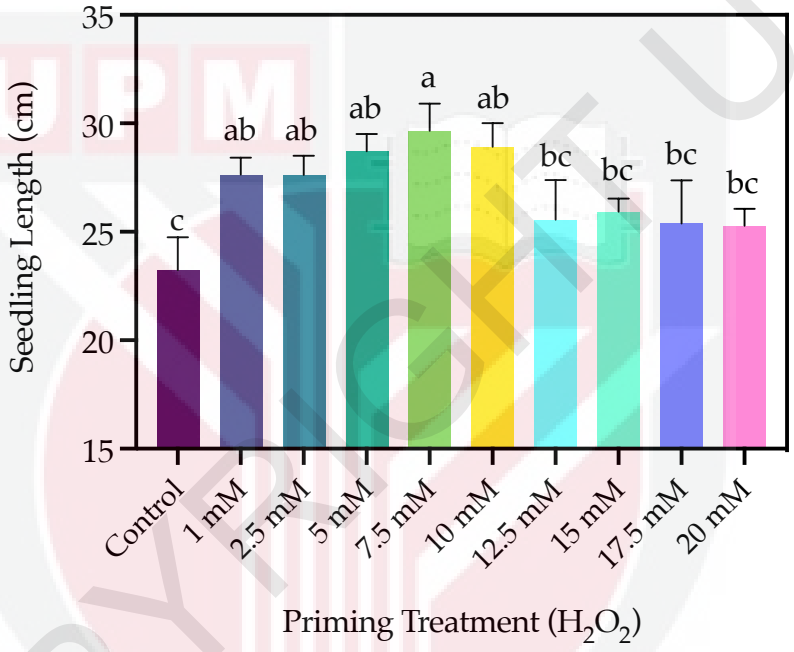

Figure 5.7: Seedling length of seeds treated with different concentration of hydrogen peroxide compared to untreated seed (control). Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

5.3.2.7 Seedling Shoot Dry Weight

Priming of sweet corn seed in different H_2O_2 concentration up to 20 mM affected seedling performance in shoot dry weight (Refer Appendix 5.8). As can be seen in Figure 5.8, the best shoot dry weight can be obtained when the seeds primed at 10 mM of H_2O_2 producing 0.032 g /seedling followed by other

seeds primed between 1 mM to 7.5 mM between 0.029 – 0.030 g /seedling which performed equally well with 10 mM seeds. However, seeds primed between 1 mM to 7.5 mM of H_2O_2 had same performance with the seeds primed between 12.5 mM to 20 mM (0.025 – 0.028 g /seedling) in which the seeds in this concentration (12.5 mM to 20 mM) were statistically equally lower with untreated seeds. Untreated seeds only produced 0.022 g of seedling which was the poorest in terms of shoot dry weight performance.

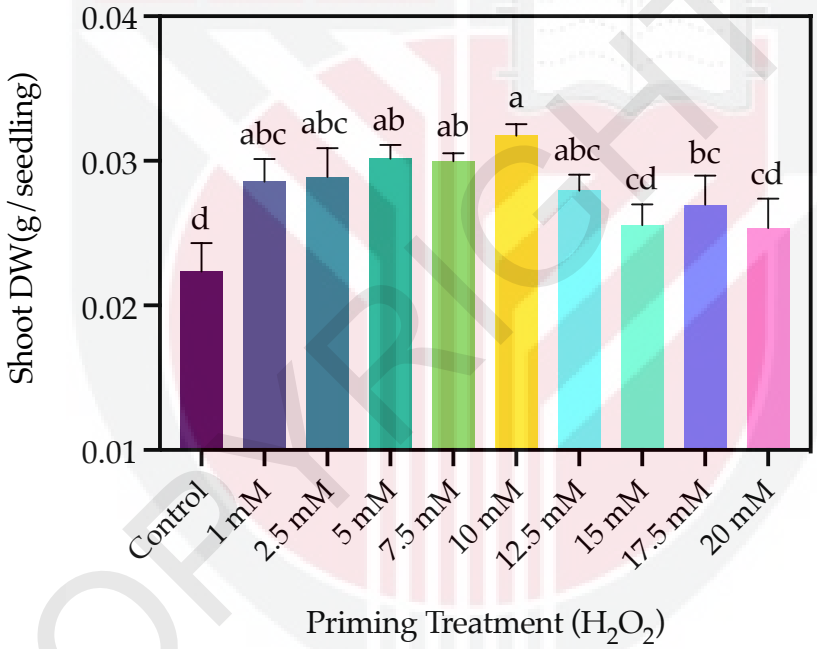

Figure 5.8: Shoot Dry Weight of seeds treated with different concentration of hydrogen peroxide compared to untreated seed (control). Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

5.3.2.8 Seedling Root Dry Weight

In contrast with parameters mentioned earlier, priming in different concentration of hydrogen peroxide showed no improvement and no significant difference was observed after ANOVA was conducted (Refer Appendix 5.9). Untreated seed produced 0.014 g of root per seedling while treatment with priming produced root ranging between 0.012 to 0.017 g per seedling.

5.3.2.9 Seedling Vigour Index

According to ANOVA (refer to Appendix 5.10), SVI of sweet corn seeds was significantly influenced by different concentrations of H_2O_2 used for 24 hour priming. The results of Tukey's Test are summarized in Figure 5.9. The highest SVI was observed in seeds primed with 10 mM H_2O_2, yielding a value of 2087.3. This was approximately 89% higher than the untreated seeds, which had an SVI of 1114.06. Seeds primed with 5 mM to 7.5 mM H_2O_2 showed similar performance, with SVI values ranging from 1956.76 to 1947.44, corresponding to a 76% to 75% increase over untreated seeds.

Seeds primed at 1 mM (1601.12) and 2.5 mM (1705.52) also performed similarly, with increases of 44% and 53%, respectively, compared to untreated seeds. Seeds primed with 12.5 mM (1516.08) and 20 mM (1386.08) showed a 36% and 24% increase, respectively, but their performance was statistically

similar to untreated seeds. The lowest SVI values were observed in seeds primed with 12.5 mM to 20 mM H_2O_2, which were statistically similar to untreated seeds, with an SVI of 1114.06, approximately 50% lower than the highest SVI recorded in this study.

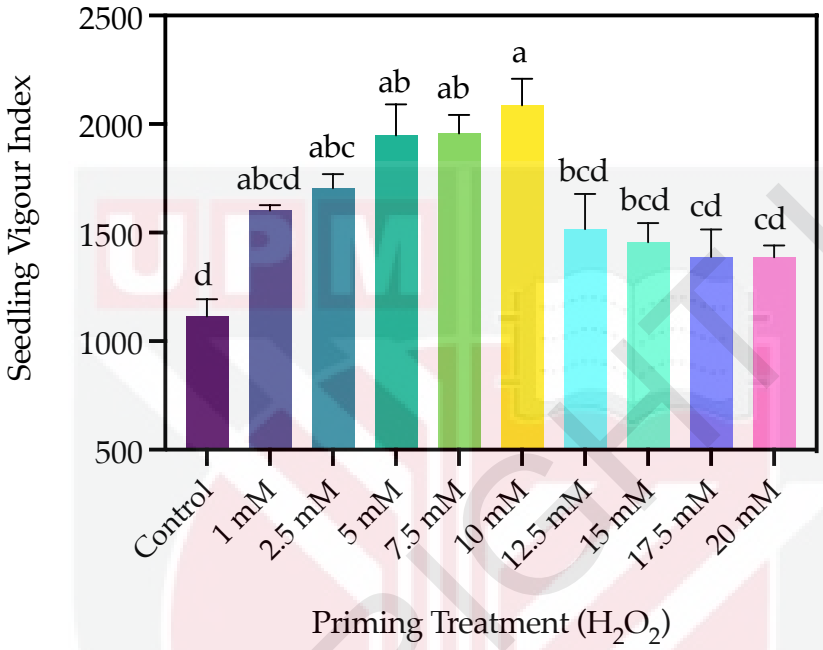

Figure 5.9: Seedling Vigour Index of seeds treated with different concentration of hydrogen peroxide compared to untreated seed (control). Means with same letter on the bars are not significantly different at P > 0.05 using Tukey Test.

5.3.2.10 Electrical Conductivity of Seed Leachate

Based on electrical conductivity (EC) test, priming treatment using different concentration of H_2O_2 for 24 hours influenced seed leachates of sweet corn seeds (Refer Appendix 5.11). The highest seed leachates were obtained from the unprime or untreated seeds which was around 106.22 $\mu S\ cm^{-1}g^{-1}$ of seeds as can be seen in Table 5.1. Priming was found to improve seed membrane integrity

in which all priming concentration between 1 mM to 20 mM had shown significant reduction in the amount of seed leachate coming out of the seeds.

Seeds primed with 10 mM H_2O_2 exhibited the highest viability and reduced seed leachate to 61.09 µS /cm /g, while seeds primed with 20 mM H_2O_2 showed the lowest EC reading of 40.13 µS /cm /g. Untreated seeds, in contrast, had a higher EC of 106.22 µS /cm /g, reflecting greater seed leachate. In terms of percentage reduction, the 10 mM H_2O_2 primed seeds showed a 42.5% reduction, while the 20 mM H_2O_2 primed seeds exhibited a 62.3% reduction in EC compared to untreated seeds.

However, based on mean comparison using Tukey's Test, no significant differences were found between the various H_2O_2 concentrations in terms of reducing seed leachates, indicating that all tested concentrations were equally effective in reducing EC levels.

5.3.2.11 Malondialdehyde Content

Lipid peroxidation as measured from MDA content extracted from the sweet corn seed sample was affected by different priming concentration of H_2O_2 at 24 hours when compared to untreated seeds (Refer Appendix 5.12). As can be seen in Table 5.1, the highest MDA content extracted from untreated seeds at 5.91 mmol /g FW, showing the highest lipid peroxidation indicating poor seed quality. Priming treatments using different concentration significantly reduced

lipid peroxidation as lower amount of MDA extracted from the primed seed sample. The lowest MDA content was extracted from 1.64 mmol /g FW, showing improvement in seed quality. Seeds between 2.5 mM to 7.5 mM of H_2O_2 also had an equal performance where priming at this concentration was ranging between 2.13 to 2.42 mmol/g FW. This was followed by the seeds primed at 1 mM (3.16 mmol /g FW) which statistically not differed with the seeds primed between 12.5 mM to 20 mM of H_2O_2 (3.49 – 4.25 mmol /g FW).

Table 5.1: Summary of analysis of variance of different priming concentration of hydrogen peroxide on biochemical activities, i.e., oxidation products and antioxidant enzymes activities.

Priming Concentration	*Oxidation Products*		*Antioxidant Enzymes Activities*		
	Electrical Conductivity of Seed Leachate µS /cm /g	Malondialdehyde (MDA) Mmol /g FW	Catalase (CAT) mmol /min /mg FW	Guaiacol Peroxidase (POD) nmol /min /mg FW	Superoxide Dismutase (SOD) unit /mg /FW
F Test	**	**	**	**	**
Control	106.22 a	5.91 a	10.78 a	12.28 a	5.33 d
1.0 mM	53.94 bc	3.16 bc	8.68 bc	7.66 e	6.42 bc
2.5 mM	55.37 bc	2.42 cd	8.37 bc	8.34 de	6.41 bc
5.0 mM	55.37 b	2.33 cd	8.89 b	9.89 bc	7.02 ab
7.5 mM	62.18 b	2.133 cd	7.63 bc	9.91 bc	7.25 ab
10.0 mM	61.09 b	1.64 d	8.07 bc	11.32 ab	7.61 a
12.5 mM	52.54 bc	3.49 bc	7.85 bc	11.35 ab	6.38 bc
15.0 mM	49.31 bc	4.19 b	7.74 bc	11.78 a	6.55 bc
17.5 mM	54.60 bc	4.31 b	7.43 c	9.66 cd	6.04 cd
20.0 mM	40.13 c	4.25 b	7.59 bc	7.22 e	5.92 cd

** indicates highly significant differences at $P \leq 0.0001$.
means with same letter vertically in each of seed quality parameters are not significantly different at $P > 0.05$ using Tukey Test.

5.3.2.12 Changes in Catalase Activity

As can be seen in Appendix 5.15, CAT enzyme activity in sweet corn seed was affected by different priming concentration of H_2O_2 when primed for 24 hours. CAT activity was observed to decrease in primed seeds compared to untreated seeds, as shown in Table 5.1. Untreated seeds had an initial CAT activity of 10.75 µmol /min /mg FW, which decreased when the seeds were primed with various concentrations of H_2O_2.

Among the primed seeds, there was no significant difference in CAT activity between seeds primed at 1 mM to 15 mM and 20 mM of H_2O_2. The highest CAT activity among the primed seeds was observed in those treated with 5 mM H_2O_2 at 8.89 µmol /min /mg FW, representing a 17.3% decrease compared to untreated seeds. In contrast, the lowest CAT activity was found in seeds primed with 17.5 mM H_2O_2 at 7.43 µmol /min /mg FW, a 30.4% reduction compared to untreated seeds.

Interestingly, seeds primed at 10 mM H_2O_2, which exhibited the highest viability, showed a CAT activity of 8.07 µmol /min /mg FW, a 24.9% reduction compared to untreated seeds, and had similar CAT activity to seeds primed at 5 mM H_2O_2.

5.3.2.13 Changes in Guaiacol Peroxidase Activity

POD showed to be influenced by H_2O_2 priming concentration up to 20 mM for 24 hours as being analysed using ANOVA in Appendix 5.13. Based on post hoc mean comparisons in Table 5.1, untreated seeds exhibited the highest POD activity at 12.22 nmol /min /mg FW. Priming with H_2O_2 generally resulted in a reduction in POD activity, with the exception of seeds primed at 10 mM, 12.5 mM, and 15 mM, where POD activity was measured at 11.32 nmol /min /mg FW, 11.36 nmol /min /mg FW, and 11.78 nmol /min /mg FW, respectively. These values represented a 7.4% to 3.6% reduction in POD activity compared to untreated seeds, but no significant differences were found between these treatments and the untreated control.

Seeds primed at 5 mM and 7.5 mM H_2O_2 showed 9.98 nmol /min /mg FW and 9.90 nmol /min /mg FW, respectively, corresponding to a 18.3% to 19.1% reduction in POD activity compared to the untreated seeds. In contrast, seeds primed at 1 mM (7.66 nmol /min /mg FW), 2.5 mM (8.34 nmol /min /mg FW), and 20 mM (7.22 nmol /min /mg FW) showed the greatest reductions in POD activity, with reductions of 37.5%, 31.3%, and 41.1%, respectively, compared to the untreated control. These three concentrations had the lowest measured POD activity. The implications of these findings will be further discussed in the discussion section

5.3.2.14 Changes in Superoxide Dismutase Activity

Priming of sweet corn seed in different concentration of H_2O_2 was found to affect the activity of SOD activity within the seeds (Refer Appendix 5.14) and means comparison was summarized in Table 5.1. As shown in the table, SOD activity increased after seeds were primed with various concentrations of H_2O_2 for 24 hours. Untreated seeds had an initial SOD activity of 5.33 unit /mg FW, which increased significantly upon priming. For example, seeds primed with 1 mM H_2O_2 exhibited a 20.5% increase in SOD activity, reaching 6.42 unit /mg FW, while seeds primed at 5 mM showed a 31.7% increase to 7.02 unit /mg FW. The highest SOD activity was observed in seeds primed at 10 mM H_2O_2, with an average of 7.61 unit /mg FW, representing a 42.8% increase compared to untreated seeds.

There was no significant difference between the 5 mM and 10 mM treatments. However, after this peak, SOD activity began to decline. Seeds primed with 20 mM H_2O_2 showed a decrease to 5.92 unit/mg FW, which represented a 10.1% decrease compared to untreated seeds. Notably, the SOD activity of seeds primed at 20 mM was not significantly different from untreated seeds.

5.4 Discussion

Seed Viability and Vigour

Sweet corn seeds are commonly stored in ATS packaging and the results in chapter 4 revealed that under this condition, placing it at 16°C was only able to keep the seed viability to around 50% after a year of storage. In this study, priming with H_2O_2 up to 20 mM for 24 hours was observed to improved seed viability with the highest at 10 mM (FGP = 69%) while priming using more than 20 mM would cause detrimental effect to seed viability of sweet corn seed. Priming with hydrogen peroxide is not new as many other crops also had improvement in terms of viability. In a rice seed, priming with 0.25% of H_2O_2, improved seed viability up to 13% increment and up to 14% rise in cotton viability after 80 mM of H_2O_2 priming (Hemalatha et al., 2017).

Previous research in other crops had shown the importance and roles of H_2O_2 contributes to promote germination in many crops. For example, Barba-Espin et al. (2010) had suggested that H_2O_2 can promote the induction of proteins related to seed germination signalling, and it works by acting as a messenger within phytohormonal setup that leads up to germination, and supplementation with H_2O_2 can fasten the germination process as being demonstrated in pea seed. On the other hand, Bahin et al. (2011) postulated that exogenous H_2O_2 helps in modulating hormonal balance between by

activation of GA signalling and biosynthesis in the seed also leading to better germination and vigour in barley seed.

All above might contribute to a better seed vigour. In this study, primed seed at 10 mM generally had better MGT, GRI, GI and CVG, indication a faster and more rapid germination, a key to uniform and quality seedling. In pepper seed invigoration, priming at 10 mM of H_2O_2 improved seeds MGT, GR AND GI while under salinity stress (Gammoudi et al., 2020).

Seedling Performance

Findings in this study showed that application of priming as low as 1 mM of H_2O_2 improved shoot dry weight and mean average of root were higher compared to untreated seeds. In this study, priming with up to 12.5 mM of H_2O_2 improved seedling shoot mass and further increase up to 20 mM had no improvement. For comparison, a study by Jira-Anunkul & Pattanagul (2020), a priming on rice seeds using H_2O_2 showed improvement in seedling length, and root and shoot dry mass when primed less than 10 mM of H_2O_2 and increase more than 15 mM will have detrimental effect on seedling performance. Another finding by Ashraf et al. (2015) showed seed priming using of H_2O_2 improved shoot and root dry mass of maize in water deficit stress study, indicating there is some role of exogenous H_2O_2 responsible in the improvement observed in all of these studies.

A research by Neto et al. (2005) showed better plant growth in terms of shoot dry mass, root dry mass and leaf area when the plant was supplemented with exogenous H_2O_2 at three days after sowing, as the study observed the increase in SOD content in plant root and leaves resulting in lower lipid peroxidation in the plant seedling. In cadmium stress rice plant, it was observed that plant shoot and root decreased, however application of H_2O_2 improved seedling growth especially on the shoot. This application also found to significantly increase glutathione level in the plant leaves (Hu et al., 2009) and there was an increase of antioxidant enzymes in rice seedling leaves (Chou et al., 2012), therefore improving seedling growth. Verma et al. (2015) also suggested that H_2O_2 production during germination would activate reserve mobilization by signalling storage organ to mobilize its reserve to promote axis growing which explain the higher shoot and root dry mass in this study. Most of these studies showed alleviation in antioxidant defence mechanism.

Seed Biochemical Activities

Plants detects alleviation of ROS and activates antioxidant mechanism to defend itself from further damages and H_2O_2 is vital as it is generated in response to various stress stimuli. H_2O_2 regulates the expressions of genes involved in ROS production and scavenging including genes encoding antioxidant enzymes to control excessive ROS accumulation (Hossain et al., 2015) and H_2O_2 priming may lead to induction of many signals imprinted in the seeds, enhancing many antioxidant stress counteractive such as

antioxidant enzymes (Paparella et al., 2015). Priming with H_2O_2 also allows plant to memorized stress-induced responses, and the respond was immediate as to activate signal stress cues beforehand (Banerjee & Roychoudhury, 2019). Finding in this study corroborates with these hypothesis as some of antioxidant content was found to be high after application of H_2O_2 priming where SOD content increased after priming.

Finding of the effect of H_2O_2 priming on different crops were varied with more of the studies showed increase in antioxidant activities. For example, in a study by Neto et al. (2005) there was an increment in SOD content on plant leaves when the maize seedling was placed in cadmium stress condition once supplemented with H_2O_2. In sunflower seeds, H_2O_2 priming recuperates antioxidant defence (Silva et al., 2020) as there was an increase of SOD, CAT and APX after priming.

It was observed that there was a reduction of CAT content once the sweet corn seed primed with H_2O_2, and there was a drop-in content of POD before it peaks back to normal between 10 mM to 15 mM of H_2O_2. A study by Jira-Anunkul & Pattanagul (2020) showed priming of H_2O_2 between 1 mM to 10 mM on rice seeds showed increase of SOD and APX enzymes activity but CAT remains the same. CAT is a key enzyme in seed recovery from ageing during the seed priming (Kibinza et al., 2011). CAT and POD work by neutralizing H_2O_2 to water and oxygen molecule while SOD provide protection by dismutate super oxide anion to H_2O_2 (Kong et al., 2015; Mhamdi et al., 2010).

In this study, H_2O_2 priming exhausted CAT and POD content as these two enzymes being used to neutralize H_2O_2, while at the same provide a beneficial effect on the seed as it activates the signal that responsible in the improvement observed in the plant such as improved seed viability, seed vigour and seedling performance including increase in SOD content mentioned earlier.

Oxidative damage during ageing can be observed by measuring seed leachate coming out from seed membrane as well as MDA, a by-product of lipid peroxidation. The benefits of priming with H_2O_2 on the antioxidant activities to counter back ROS damage in the seeds can be seen as there was a reduction MDA content and seed leachates during electrical conductivity test. All priming concentration up to 20 mM of H_2O_2 showed lowered amount of damage measured with the lowest at 10 mM of H_2O_2 (1.64 mmol / g FW). Other priming study using H_2O_2 also showed reduction of MDA in crops such as wheat (Hameed & Iqbal, 2014), rice (Jira-Anunkul & Pattanagul, 2020), maize (Ashraf et al., 2015), sunflower (Silva et al., 2020) among all. In maize seedling, 10 mM pre-treatment was found to reduce MDA level of primed seedling (Terzi et al., 2014) which had similar finding in this research where 10 mM of H_2O_2 had the lowest MDA content measured. Habib et al. (2020) stated that, exogenous H_2O_2 priming helped in regulating key enzymatic antioxidant enzymes which neutralized many ROS overproduction and reduced further oxidative impairments reducing lipid peroxidation.

Lesser seed leachate was measured in the seeds once primed is one of the indicator of membrane repair activity occurring within the seed (Khaliq et al., 2015). In this study, seed leachate reduced from 106.22 µS /cm /g in untreated seed to 61.09 µS /cm /g in 10 mM H_2O_2 showing how H_2O_2 helps in signalling repair mechanism within the seeds.

5.5 Conclusion

Seed ageing is an inevitable process occurring during seed storage and some actions are needed to improve its quality before sowing to obtain a good seedling establishment. In our study, preliminary results showed that priming of sweet corn seed between 1 to 20 mM of H_2O_2 helps in improving seed viability while priming between 20 to 100 mM H_2O_2 would cause detrimental effects on seed viability.

Further, the study showed that aged sweet corn seeds can be invigorated using H_2O_2 priming at 10 mM concentration for 24 hours before germination. Priming in this concentration, improved seed viability from 48% (untreated) to 69% (10 mM H_2O_2). Priming at this concentration help to improve seed vigour such lower MGT, higher GR, GRI and CVG, longer seedling, better shoot dry weight and high SVI. Introduction of exogenous H_2O_2 during priming increased the activation of antioxidant enzymes such as SOD thus improving seed condition, as seen in reduction of lipid peroxidation through lower MDA content and seed membrane leachate.

CHAPTER 6

SUMMARY, CONCLUSION AND RECOMMMENDATION

The increase in demand of sweet corn requires improvement in seed production to allow large scale cultivation. This research was conducted to address problems hindering the large production of sweet corn seed. Seed production of sweet corn had been impeded by many seed quality problem such as low germination, poor vigour and seedling establishment as well as low storability as reported by many scholars. Time of harvest is important in determining quality of seed as early harvested seed tend to have higher moisture, making the seeds unsuitable for storage. On the other hand, late harvesting had been known to cause many loss of quality of seed with longer exposure to pest and disease attack in the field. Hence, the right time must be properly identified to make sure quality seed can be obtained. GSH11005Y is a new hybrid of *shrunken2* sweet corn which is soon to be released to public, however, there have been no study had been conducted in identifying the right time to harvest quality seed of this particular sweet corn.

Post-harvest drying is required because sweet corn seed usually harvested at high moisture content, and it must be reduced to 8% in order to improve its storability. Post-harvest drying requires seeds to be exposed to different temperature condition, eliminating the moisture from the seed. Prior to the study, several papers had shown improvement in sweet corn starch content

went to seed subjected at two different condition either at low temperature or room temperature. There were changes in sweet corn seed starch and sucrose content, with higher temperature (room temperature) causing seed starch to increase and reducing seed sucrose content. These studies only limited to room temperature, and whether higher temperature, for example during post-harvest drying for seed production, may affect seed starch and sucrose content.

In first study (Chapter 3), the two factors were evaluated to know if seed quality of sweet corn can be improved by harvesting the seed at certain maturity stages and followed by post-harvest drying at different temperature would affect its starch and sucrose content, resulting in better storability of sweet corn seed. The finding showed that GSH11005Y sweet corn seed had reached it maximum dry weight at 29 DAP, but its dry weight reduced and maintain afterward, indicating that physiological maturity had been obtained at the point of harvest, in this case between 29 to 41 DAP. Subsequent drying showed that the fastest way to reduce seed moisture was at 45˚C, followed 40˚C and 35˚C. However, it is important to note that, higher seed moisture affected seed coat, as increase in seed leachate was observed when seeds dried at higher temperature. Germination test was conducted and seed qualities parameters was measured. Result showed that in initial performance, seed viability was nor affected by neither factor, but it was affecting seed vigour and seedling performance. Harvesting time affected seed vigour with early harvested seed (between 29 – 35 DAP) had better qualities such as higher CVG,

and MGT, hence early, faster and rapid germination can be obtained from this seeds, in contrast with late harvested seed (38-41 DAP). Drying temperature is more stand out in seedling performance where the seeds dried between 35˚C and 40˚C produced better seedling, as measured from seedling length, dry mass and SVI. Although past studies had shown increase in starch content at room temperature than cool temperature, certainly this wasn't the case when higher temperature (between 35˚C to 45˚C) was used. There was reduction in starch and increase in sucrose content observed in this study. There was lesser reduction of starch in early harvested seed (29-35 DAP) when it subjected to 35˚C and 40˚C, which is favourable for storage. CAT was observed higher in 29-35 DAP seeds while POD content was higher in 35 to 38 DAP seeds. Drying at 40˚C would result in higher spike of POD activity in combating in oxidative damage, as can be seen in MDA content.

In second study of Chapter 4, focus was given on storability of sweet corn seed. Temperature and oxygen level are two factors that affect seed longevity during seed storage. In this study, air was removed using vacuum sealer machine, resulting in three different packaging level, FV, PV and ATS packaging and then stored in two different environment temperature, 16°C and 26±3°C room condition.

Finding in the second study showed that by limiting the amount of oxygen level in packaging types, seeds storability can be maintained more than 80% even after a year of storage. Interestingly, if the seeds stored in vacuum

packaging can maintained seed viability in 26±3°C room temperature as good as seeds stored in cool room up to 6 MAS. Seed germination of FV and PV seed was between 80.5 to 88.5% with no significant difference between cool room and 26±3°C. However, for long term storage, only seeds stored in cool room temperature maintain higher viability. In comparison, seed germination at cool room was more than 85% while in 26±3°C it dropped tremendously to 33%.

At the end of 12 MAS, seeds stored in FV in 16°C had better performance compared to 26±3°C room temperature. Storing seed in this condition would reduce MGT to only 4.68 days, with highest GI at 331, and rapid and uniform emergence (CVG at 20.07). Seed stored in this condition also had better SVI, longer seedling length and higher dry mass (shoot and root). The storability of seed under this condition was better due to higher antioxidant mechanism activities as observed in SOD (4.43 unit /mg /FW), POD (22.17 nmol /min /mg FW) and CAT (9.90 µmol /min mg FW) content.

Seed deterioration is inevitable process and it is irreversible. However, priming treatment can help in reinvigorating those deteriorated seed. Several studies had shown promising result of using H_2O_2 compound as priming agent on many crops but it yet to be tested on sweet corn seeds, hence the study three in Chapter 5. Through preliminary study it was observed that priming up to 20 mM of H_2O_2 did improve seed viability of sweet corn. Different concentration of H_2O_2 between 0 to 20 mM of H_2O_2 was then

selected, and seed qualities such as seed viability, seed vigour, seedling performance and antioxidant activities response were measured. Result showed than priming between 5 mM to 10 mM provide the best germination response with maximum germination obtained at 10 mM of H_2O_2 at 69%, compared to control at just 48%.

Based on seed vigour parameters, seeds primed at 10 mM H_2O_2 consistently better compared to other priming treatment. It only needed 4 days for seed to germinate compared to control which required 5 days. GRI was also peaking at 291 in primed seed of 10 mM H_2O_2, two times better than untreated seed. Seedling parameters showed that priming between 1 mM to 12.5 mM had an equal performance and it improved seedling length and shoot dry weight. All priming treatment between 1 mM to 20 mM also showed a reduction in measurement of seed leachate showing that priming using H_2O_2 provide some repair mechanism to the seed membrane integrity. This can be seen in MDA content where there was reduction in lipid peroxidation of seed, where MDA content of primed seed at 10 mM H_2O_2 was only at 1.64 mmol /g FW compared to untreated seed where MDA accumulated at 5.91 mmol /g FW. The improvement in seed viability, seed vigour, seedling performance as well as repairing mechanism in seed membrane can be attributed to antioxidant mechanism such as POD, SOD and CAT. Priming with H_2O_2 did improve POD and SOD activities within the seeds with lesser activity of CAT was observed. This lesser amount of CAT was due to response of this antioxidant enzyme to

neutralize exogenous H_2O_2 from our priming concentration, hence the reduction observed.

Based on all finding obtained from this thesis, conclusion and recommendation can be drawn as following:

1. Seed quality of sweet corn is affected by the timing of when seeds are harvested, and the temperature used during post-harvest drying. For immediate use, GSH11005Y seeds can be harvested as early as 29 DAP, but for storage, the recommended time to harvest the seed is at 35 DAP as it has the right balance between seed viability, vigour and seedling performance.
2. Post-harvest drying is very important step to prolong sweet corn seed storability. It is suggested that seed of sweet corn to be dried at 40˚C as it can reduce seed moisture to safe level of 8% faster compared to 35˚C but at the same maintaining seed membrane integrity that was damaged at 45˚C. Drying at temperature between 35˚C to 45˚C however reduced seed starch, but drying between 35˚C to 40˚C had lesser reduction and drying at 40˚C proven is better with higher POD activities which help to mitigate deleterious effect of ROS during storability.
3. Both oxygen level and environment temperature play an important roles in maintaining storability of sweet corn. Modification of packaging by removing air from the package itself through vacuum can

be helpful in prolonging seed viability. Storing seed in vacuum condition (-0.09 to -0.1 mPa) can maintain seed viability to more than 80% after year of storage.

4. For storing below than 6 months, it is sufficient to store sweet corn seed at 26±3°C room temperature, provided that seeds are stored in vacuum condition. However, for long term storage, cool environment such as in cool room at 16°C is important in maintaining seed quality.
5. Deteriorated seed can be reinvigorated using priming treatment. Priming with H_2O_2 is an efficient priming agent in invigorating deteriorated sweet corn seed. Priming sweet corn seed at 10 mM of H_2O_2 concentration is proven to improve seed viability, seed vigour as well seedling establishment of sweet corn.

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APPENDICES

Appendix 3.1: Analysis of variance of seed fresh weight of seeds at before drying.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	21.17208	5.29302	18.72	<.0001	**
Error	15	4.23897	0.282598			
Total	19	25.4110				

** indicates highly significant differences at P ≤ 0.0001

Appendix 3.2: Analysis of variance of glucose content of seeds at before drying.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	7.96776	1.99194	11.43	<.0001	**
Error	10	1.7416	0.17416			
Total	14					

** indicates highly significant differences at P ≤ 0.0001

Appendix 3.3: Analysis of variance of sucrose content of seeds at before drying.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	10	83.9295	20.98239	30.10	<.0001	**
Error	4	6.96915	0.696915			
Total	14	90.8987				

** indicates highly significant differences at P ≤ 0.0001

Appendix 3.4: Analysis of variance of starch content of seeds at before drying.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	6.69885	1.674714	0.679	0.621442	ns
Error	10	24.63139	2.4631396			
Total	14					

ns indicates no significant differences

Appendix 3.5: Analysis of variance of seed moisture content of seeds at before drying.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	778.7880	194.6970	37.92	<.0001	**
Error	15	77.00494	5.133663			
Total	19	855.7930				

** indicates highly significant differences at P ≤ 0.0001

Appendix 3.6: Analysis of variance of seed dry weight of seeds at before drying.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	15.76137	3.9403425	10.50	<.0001	**
Error	15	5.625525	0.375035			
Total	19	21.38689				

** indicates highly significant differences at P ≤ 0.0001

Appendix 3.7: Analysis of variance of 100 seed weight post-drying.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	20.3114	5.077493	13.30	<.0001	**
Drying Temperature	2	8.49359	4.246459	11.14	<.0001	*
MS × DT	8	7.71039	0.963733	2.528	0.0003	*
Error	45	8.44476	0.381269			
Total	59	44.957				

** indicates highly significant differences at P ≤ 0.0001
* indicates significant differences at P ≤ 0.05

Appendix 3.8: Analysis of variance of final germination percentage of seeds before storage.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	17.6000	4.4000	1.03	0.4016	ns
Drying Temperature	2	4.8000	2.4000	0.56	0.5737	ns
MS × DT	8	35.2000	4.40000	1.03	0.4272	ns
Error	45	192.0000	4.2667			
Total	59	249.6000				

ns indicates no significant differences

Appendix 3.9: Analysis of variance of mean germination time of seeds before storage.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	0.9716	0.2429	5.70	0.0008	**
Drying Temperature	2	0.0462	0.0231	0.54	0.5853	ns
MS × DT	8	0.2543	0.0318	0.75	0.6505	ns
Error	45	1.9162	0.0426			
Total	59	3.1883				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.10: Analysis of variance of germination rate index of seeds before storage.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	45.1123	11.2780	4.44	0.0041	**
Drying Temperature	2	2.7556	1.3777	0.54	0.5848	ns
MS × DT	8	10.4401	1.3051	0.51	0.8393	ns
Error	45	114.1807	2.5371			
Total	59	172.4896				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.11: analysis of variance germination index of seeds before storage.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	7465.066	1866.2667	3.88	0.0086	**
Drying Temperature	2	488.5333	244.2667	0.51	0.6054	ns
MS × DT	8	2274.133	284.2667	0.59	0.7803	ns
Error	45	21660.00	481.3333			
Total	59	31887.73				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.12: Analysis of variance of coefficient velocity of germination of seeds before storage.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	43.8150	10.9538	5.44	0.0012	**
Drying Temperature	2	2.4579	1.2289	0.61	0.5476	ns
MS × DT	8	11.6491	1.4561	0.72	0.6702	ns
Error	45	90.6205	2.0137			
Total	59	148.5427				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.13: Analysis of variance of seedling vigour index of seeds before storage.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	87228.77	21807.1938	0.71	0.5894	ns
Drying Temperature	2	162653.4	81326.7353	2.65	0.0318	*
MS × DT	8	245627.6	30703.4577	1.00	0.4496	ns
Error	45	1382085.	30713.016			
Total	59	1877595.				

* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 3.14: Analysis of variance of seedling length of seeds before storage.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	10.13107	2.53276833	0.83	0.5120	ns
Drying Temperature	2	12.87316	6.43658167	2.11	0.0325	*
MS × DT	8	26.03898	3.25487333	1.07	0.4013	ns
Error	45	136.9860	3.0441350			
Total	59	186.0292				

* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 3.15: Analysis of variance of seedling root dry weight of seeds before storage.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	0.000289	0.00007230	1.13	0.3562	ns
Drying Temperature	2	0.000397	0.00019853	3.09	0.0452	*
MS × DT	8	0.000103	0.00001290	0.20	0.9893	ns
Error	45	0.002889	0.00006420			
Total	59	0.003678				

* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 3.16: Analysis of variance of seedling shoot dry weight of seeds before storage.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	0.000089	0.00002241	5.08	0.2018	ns
Drying Temperature	2	0.000040	0.00002023	4.58	0.0154	*
MS × DT	8	0.000037	0.00000475	1.08	0.3968	ns
Error	45	0.000198	0.00000441			
Total	59	0.000366				

* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 3.17: Analysis of variance of seed leachate of seeds at post-drying.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	2817.998	704.4995	36.66	<.0001	**
Drying Temperature	2	705.6282	176.40705	18.36	<.0001	**
MS × DT	8	224.9977	28.124471	0.012	0.2151	ns
Error	45	2194.252	48.761167			
Total	59	4531.62				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.18: Analysis of variance of glucose content of seeds post-drying.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	5.618349	1.404587	14.68	<.0001	**
Drying Temperature	3	12.79016	4.263388	44.54	<.0001	**
Block	2	0.082701	0.041351	0.43	0.6523	ns
MS × DT	12	4.153911	0.346159	3.62	0.0012	**
Error	38					
Total	59					

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.19: Analysis of variance of sucrose content of seeds at post-drying.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	11.73065	2.932663	2.93	0.0329	*
Drying Temperature	3	476.3276	158.7759	158.9	<.0001	**
Block	2	3.524488	1.762244	1.76	0.1852	ns
MS × DT	12	41.89904	3.491586	3.49	0.0016	**
Error	38	3.79702	0.099921			
Total	59					

** indicates highly significant differences at P ≤ 0.0001
* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 3.20: Analysis of variance of starch content of seeds at post-drying.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	88.62	22.155	18.63	<.0001	**
Drying Temperature	3	272.2	90.73333	76.31	<.0001	**
Block	2	22.15	11.075	9.25	0.0126	ns
MS × DT	12	134.99	11.24917	9.45	0.0143	*
Error	38	45.21	1.189737			
Total	59					

** indicates highly significant differences at P ≤ 0.0001
* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 3.21: Analysis of variance of catalase activity of seeds at post-drying.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	12.85538	3.21384582	3.01	0.0348	**
Drying Temperature	2	6.935417	3.46770897	3.25	0.0538	ns
Block	2	13.52764	6.76382070	6.34	0.0054	**
MS × DT	8	5.231319	0.65391493	0.61	0.7595	ns
Error	28	29.87925	1.06711642			
Total	44	68.42902				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.22: Analysis of variance of guaiacol peroxidase activity of seeds at post-drying.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	13869.64	3467.41091	274.0	<.0001	**
Drying Temperature	2	10752.25	5376.12844	424.8	<.0001	**
Block	2	4.55706	2.27853	0.18	0.8362	ns
MS × DT	8	3308.698	413.58726	32.69	<.0001	**
Error	28	354.2928	12.65332			
Total	44	28289.44				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.23: Analysis of variance of malondialdehyde content of seeds at post-drying.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	2.288424	0.57210613	10.95	<.0001	**
Drying Temperature	2	0.478750	0.23937526	4.58	0.0190	*
Block	2	0.080441	0.04022050	0.77	0.4726	ns
MS × DT	8	0.173644	0.02170559	0.42	0.9019	ns
Error	28	1.462847	0.05224456			
Total	44	4.484108				

** indicates highly significant differences at P ≤ 0.0001
* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 3.24: Analysis of variance of final germination percentage of seeds at 3 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	27.7333	6.9333	0.96	0.4371	ns
Drying Temperature	2	19.2000	9.6000	1.33	0.2738	ns
MS × DT	8	103.4667	12.9333	1.80	0.1029	ns
Error	45	324.0000	7.2000			
Total	59	474.4000				

ns indicates no significant differences

Appendix 3.25: Analysis of variance of final germination percentage of seeds at 6 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	27.7333	6.9333	1.28	0.2925	ns
Drying Temperature	2	59.2000	29.6000	5.46	0.0075	**
MS × DT	8	47.4667	5.9333	1.09	0.3848	ns
Error	45	244.0000	5.4222			
Total	59	378.4000				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.26: Analysis of variance of final germination percentage of seeds at 9 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	149.3333	37.3333	1.63	0.1837	ns
Drying Temperature	2	241.6000	120.8000	5.27	0.0088	**
MS × DT	8	113.0667	14.1333	0.62	0.7595	ns
Error	45	1032.000	22.9333			
Total	59	1536.000				

** indicates highly significant differences at P ≤ 0.0001
* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 3.27: Analysis of variance of final germination percentage of seeds at 12 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	419.7333	104.9333	12.43	<.0001	**
Drying Temperature	2	462.9333	231.4667	27.41	<.0001	**
MS × DT	8	289.0667	36.1333	4.28	0.0007	**
Error	45	380.000	8.4444			
Total	59	1551.733				

** indicates highly significant differences at P ≤ 0.0001

Appendix 3.28: Analysis of variance of mean germination time of seeds at 3 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	1.4049	0.3512	7.72	<.0001	**
Drying Temperature	2	0.2321	0.1160	2.55	0.0893	ns
MS × DT	8	0.5115	0.0639	1.41	0.2205	ns
Error	45	2.0475	0.0455			
Total	59	4.1959				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.29: Analysis of variance of mean germination time of seeds at 6 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	0.8291	0.2073	6.20	0.0005	**
Drying Temperature	2	0.2059	0.1029	3.08	0.0557	ns
MS × DT	8	0.3574	0.0446	1.34	0.2504	ns
Error	45	1.5040	0.0334			
Total	59	2.8963				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.30: Analysis of variance of mean germination time of seeds at 9 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	0.5552	0.1388	3.70	0.0109	*
Drying Temperature	2	0.2013	0.1006	2.68	0.0792	ns
MS × DT	8	0.1177	0.0147	0.39	0.9190	ns
Error	45	1.6875	0.0375			
Total	59	2.5617				

* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 3.31: Analysis of variance of mean germination time of seeds at 12months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	0.1565	0.0391	0.19	0.9439	ns
Drying Temperature	2	0.3326	0.1663	0.80	0.4577	ns
MS × DT	8	0.3902	0.0487	0.23	0.9826	ns
Error	45	9.4101	0.2091			
Total	59	10.2895				

ns indicates no significant differences

Appendix 3.32: Analysis of variance of germination rate index of seeds at 3 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	39.1348	9.7837	7.20	0.0001	**
Drying Temperature	2	11.6167	5.8083	4.28	0.0199	*
MS × DT	8	23.1984	2.8998	2.13	0.0518	ns
Error	45	61.1230	1.3582			
Total	59	135.0728				

** indicates highly significant differences at P ≤ 0.0001
* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 3.33: Analysis of variance of germination rate index of seeds at 6 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	25.5587	6.3896	6.51	0.0003	**
Drying Temperature	2	5.6835	2.8417	2.89	0.0658	ns
MS × DT	8	5.9836	0.7479	0.76	0.6378	ns
Error	45	44.1974	0.9821			
Total	59	81.4232				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.34: Analysis of variance of germination rate index of seeds at 9 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	6.8392	1.7098	0.77	0.5489	ns
Drying Temperature	2	29.8761	14.9380	6.75	0.0027	**
MS × DT	8	9.2018	1.15023	0.52	0.8353	ns
Error	45	99.5998	2.2133			
Total	59	145.5169				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.35: Analysis of variance of germination rate index of seeds at 12months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	32.7175	8.1793	0.62	0.6497	ns
Drying Temperature	2	71.1223	35.5611	2.70	0.0780	ns
MS × DT	8	53.8761	6.7345	0.51	0.8414	ns
Error	45	592.4682	13.1659			
Total	59	750.1842				

ns indicates no significant differences

Appendix 3.36: Analysis of variance of germination index of seeds at 3 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	11990.40	2997.60	6.37	0.0004	**
Drying Temperature	2	2884.80	1442.40	3.07	0.0564	ns
MS × DT	8	7251.20	906.40	1.93	0.0790	ns
Error	45	21160.00	470.22			
Total	59	43286.40				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.37: Analysis of variance of germination index of seeds at 6 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	9236.26	2309.06	6.08	0.0005	**
Drying Temperature	2	2198.93	1099.46	2.90	0.0656	ns
MS × DT	8	2683.73	335.46	0.88	0.5375	ns
Error	45	17080.00	379.55			
Total	59	31198.93				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.38: Analysis of variance of germination index of seeds at 9 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	3052.266	763.067	1.08	0.3759	ns
Drying Temperature	2	9232.533	4616.267	6.56	0.0032	**
MS × DT	8	2754.133	344.266	0.49	0.8576	ns
Error	45	31684.00	704.088			
Total	59	46722.93				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.39: Analysis of variance of germination index of seeds at 12months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	7422.400	1855.600	0.81	0.5270	ns
Drying Temperature	2	19662.40	9831.200	4.28	0.0199	*
MS × DT	8	12337.60	1542.200	0.67	0.7140	ns
Error	45	103416.0	2298.133			
Total	59	142838.4				

* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 3.40: Analysis of variance of coefficient velocity of germination of seeds at 3 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	41.1443	10.2860	8.19	<.0001	**
Drying Temperature	2	8.6329	4.3164	3.44	0.0408	*
MS × DT	8	15.9901	1.9988	1.59	0.1543	ns
Error	45	56.5170	1.2559			
Total	59	122.2851				

** indicates highly significant differences at P ≤ 0.0001
* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 3.41: Analysis of variance of coefficient velocity of germination of seeds at 6 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	23.22014	5.80503681	6.20	0.0005	**
Drying Temperature	2	5.33959	2.66979635	2.85	0.0683	ns
MS × DT	8	9.484160	1.18552004	1.27	0.2853	ns
Error	45	42.15941	0.93687578			
Total	59	80.20331				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.42: Analysis of variance of coefficient velocity of germination of seeds at 9 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	15.21170	3.80292593	3.75	0.0103	*
Drying Temperature	2	5.959560	2.97978032	2.94	0.0634	ns
MS × DT	8	3.741103	0.46763798	0.46	0.8771	ns
Error	45	45.68433	1.01520741			
Total	59	70.59670				

* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 3.43: Analysis of variance of coefficient velocity of germination of seeds at 12 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	11.60728	2.90182199	0.26	0.8992	ns
Drying Temperature	2	16.44601	8.22300711	0.75	0.4785	ns
MS × DT	8	23.06178	2.88272348	0.26	0.9747	ns
Error	45	493.8229	10.9738440			
Total	59	544.9380				

ns indicates no significant differences

Appendix 3.44: Analysis of variance of seedling vigour index of seeds at 3 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	253012.2	63253.0636	2.38	0.0658	ns
Drying Temperature	2	187734.9	93867.4574	3.53	0.0377	**
MS × DT	8	675563.6	84445.4593	3.18	0.0061	ns
Error	45	1196456	26587.914			
Total	59	2312766				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.45: Analysis of variance of seedling vigour index of seeds at 6 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	169616.4	42404.1212	1.61	0.1891	ns
Drying Temperature	2	415264.9	207632.462	7.87	0.0012	**
MS × DT	8	227434.	28429.3348	1.08	0.3961	ns
Error	45	1187710.	26393.567			
Total	59	2000026				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.46: Analysis of variance of seedling vigour index of seeds at 9 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	244465.5	61116.380	1.49	0.2215	ns
Drying Temperature	2	1416777	708388.771	17.26	<.0001	**
MS × DT	8	343968	42996.022	1.05	0.4161	ns
Error	45	1847257	41050.156			
Total	59	3852468				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.47: Analysis of variance of seedling vigour index of seeds at 12 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	1160267	290066.937	6.65	0.0003	**
Drying Temperature	2	936345	468172.522	10.73	0.0002	**
MS × DT	8	864842	108105.316	2.48	0.0256	**
Error	45	1962761	43616.921			
Total	59	4924216				

** indicates highly significant differences at P ≤ 0.0001

Appendix 3.48: Analysis of variance of seedling length of seeds at 3 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	27.41644	6.85411000	3.06	0.0258	*
Drying Temperature	2	11.01828	6.50914000	2.46	0.0367	*
MS × DT	8	45.34192	5.66774000	2.53	0.0229	ns
Error	45	100.7141	2.2380911			
Total	59	184.4907				

* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 3.49: Analysis of variance of seedling length of seeds at 6 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	14.07576	3.51894000	1.41	0.2459	ns
Drying Temperature	2	18.81412	9.40706000	3.77	0.0306	*
MS × DT	8	28.49668	3.56208500	1.43	0.2114	ns
Error	45	112.2709	2.4949089			
Total	59	173.6574				

* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 3.50: Analysis of variance of seedling length of seeds at 9 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	10.67684	2.66921000	1.82	0.1408	ns
Drying Temperature	2	66.56857	33.2842866	22.74	<.0001	**
MS × DT	8	25.25956	3.15744500	2.16	0.0494	ns
Error	45	65.85270	1.4633933			
Total	59	168.3576				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.51: Analysis of variance of seedling length of seeds at 12 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	32.26720	8.06680000	2.65	0.0453	ns
Drying Temperature	2	17.57769	8.78884667	2.89	0.0661	*
MS × DT	8	47.43144	5.92893000	1.95	0.0758	ns
Error	45	136.9346	3.0429911			
Total	59	234.2109				

* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 3.52: Analysis of variance of seedling root dry weight of seeds at 3 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	0.000144	0.00003615	3.78	0.0098	**
Drying Temperature	2	0.000122	0.00006115	6.39	0.0036	**
MS × DT	8	0.000048	0.00000602	0.63	0.7488	ns
Error	45	0.000430	0.00000956			
Total	59	0.000745				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.53: Analysis of variance of of seedling root dry weight of seeds at 6 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	0.000228	0.00005710	4.62	0.0033	**
Drying Temperature	2	0.000044	0.00002237	1.81	0.1756	ns
MS × DT	8	0.000165	0.00002071	1.67	0.1311	ns
Error	45	0.000556	0.00001237			
Total	59	0.000995				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.54: Analysis of variance of of seedling root dry weight of seeds at 9 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	0.000136	0.00003423	2.50	0.0356	*
Drying Temperature	2	0.000051	0.00002571	1.88	0.1646	ns
MS × DT	8	0.000113	0.00001424	1.04	0.4208	ns
Error	45	0.000615	0.00001369			
Total	59	0.000918				

* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 3.55: Analysis of variance of of seedling root dry weight of seeds at 12months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	0.000198	0.00004969	10.60	<.0001	**
Drying Temperature	2	0.000088	0.00004424	9.44	0.0004	**
MS × DT	8	0.000073	0.00000916	1.95	0.0750	ns
Error	45	0.000210	0.00000469			
Total	59	0.000571				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.56: Analysis of variance of of seedling root dry weight of seeds at 3 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	0.000053	0.00001349	2.01	0.1098	ns
Drying Temperature	2	0.000031	0.00001585	2.36	0.1063	ns
MS × DT	8	0.000057	0.00000713	1.06	0.4075	ns
Error	45	0.000302	0.00000673			
Total	59	0.000445				

ns indicates no significant differences

Appendix 3.57: Analysis of variance of seedling shoot dry weight of seeds at 6 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	0.000046	0.00001171	2.08	0.0987	ns
Drying Temperature	2	0.000028	0.00001412	2.51	0.0924	ns
MS × DT	8	0.000071	0.00000896	1.59	0.1536	ns
Error	45	0.000253	0.00000562			
Total	59	0.000399				

ns indicates no significant differences

Appendix 3.58: Analysis of variance of seedling shoot dry weight of seeds at 9 months.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	0.000133	0.00003330	3.77	0.0100	**
Drying Temperature	2	0.000095	0.00004781	5.41	0.0078	**
MS × DT	8	0.000131	0.00001640	1.86	0.0913	ns
Error	45	0.000397	0.00000884			
Total	59	0.000757				

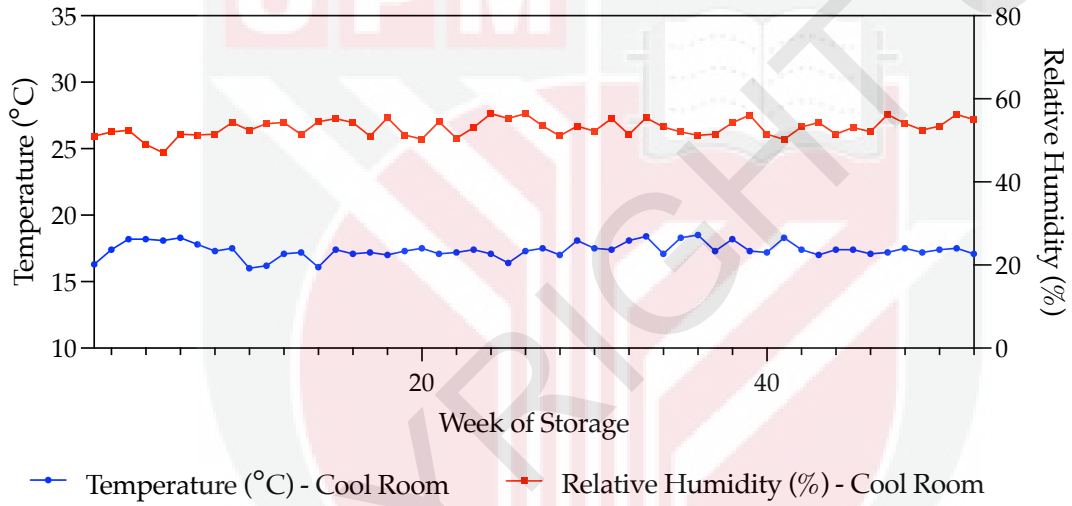
** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 3.59: Analysis of variance of seedling shoot dry weight of seeds at 12 months.

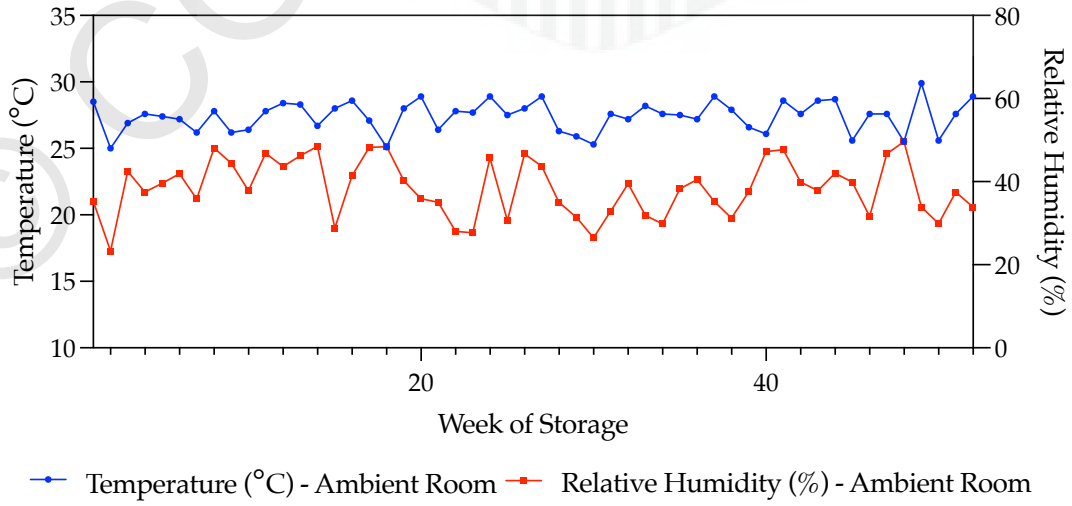
Source of Variation	DF	SS	MS	F	Pr > F	Notes
Maturity Stages	4	0.000146	0.00003657	4.87	0.0024	**
Drying Temperature	2	0.000006	0.00000345	0.46	0.6344	ns
MS × DT	8	0.000083	0.00001040	1.39	0.2282	ns
Error	45	0.000337	0.00000750			
Total	59	0.000573				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.1: Room temperature and relative humidity of sweet corn storage room from December 9th, 2020, to December 10th, 2021, in Cool Room of Seed Laboratory, MARDI, Serdang.



Appendix 4.2: Room temperature and relative humidity of sweet corn storage room from December 9th, 2020, to December 10th, 2021, in Working Area of Seed Laboratory, MARDI, Serdang.



Appendix 4.3: Analysis of variance of final germination percentage of seeds at 3 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	138792.058	69396.0288	0.80	0.4651	ns
Storage Temp.	1	154600.022	154600.022	1.78	0.1988	ns
PT × ST	2	769.2096	384.6048	0.00	0.9956	ns
Error	18	1563189.23	86843.847			
Total	23	1857350.52				

ns indicates no significant differences

Appendix 4.4: Analysis of variance of final germination percentage of seeds at 6 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	4163242.94	2081621.47	26.05	<.0001	**
Storage Temp.	1	240256.068	240256.068	3.01	0.1000	ns
PT × ST	2	426197.748	213098.874	2.67	0.0967	ns
Error	18	1438252.95	79902.942			
Total	23	6267949.71				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.5: Analysis of variance of final germination percentage of seeds at 9 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	1318050.70	659025.35	14.87	0.0002	**
Storage Temp.	1	13174387.4	13174387.4	297.3	<.0001	**
PT × ST	2	251610.86	125805.43	2.84	0.0848	ns
Error	18	797609.86	44311.66			
Total	23	15541658.9				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.6: Analysis of variance of final germination percentage of seeds at 12 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	2470769.80	1235384.90	39.60	<.0001	**
Storage Temp.	1	21833321.4	21833321.4	699.9	<.0001	**
PT × ST	2	197774.29	98887.15	3.17	0.0662	ns
Error	18	561523.02	31195.72			
Total	23	25063388.5				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.7: Analysis of variance of mean germination time of seeds at 3 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	0.02837628	0.01418814	0.23	0.7930	ns
Storage Temp.	1	0.03792125	0.03792125	0.63	0.4384	ns
PT × ST	2	0.07878173	0.03939087	0.65	0.5327	ns
Error	18	1.08695801	0.06038656			
Total	23	1.23203727				

ns indicates no significant differences

Appendix 4.8: Analysis of variance of mean germination time of seeds at 6 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	0.49248854	0.24624427	5.50	0.0137	*
Storage Temp.	1	0.23877867	0.23877867	5.33	0.0331	*
PT × ST	2	0.30965294	0.15482647	3.46	0.0537	ns
Error	18	0.80656524	0.04480918			
Total	23	1.84748539				

* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 4.9: Analysis of variance of mean germination time of seeds at 9 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	0.03637803	0.01818901	1.12	0.3474	ns
Storage Temp.	1	10.4572909	10.4572909	644.9	<.0001	**
PT × ST	2	0.02287749	0.01143875	0.71	0.5070	ns
Error	18	0.29183718	0.01621318			
Total	23	10.8083836				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.10: Analysis of variance of mean germination time) of seeds at 12 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	2.46770795	1.23385398	28.47	<.0001	**
Storage Temp.	1	1.35733863	1.35733863	31.32	<.0001	**
PT × ST	2	1.13663649	0.56831824	13.12	0.0003	**
Error	18	0.77997710	0.04333206			
Total	23	5.74166018				

** indicates highly significant differences at P ≤ 0.0001

Appendix 4.11: Analysis of variance of germination index of seeds at 3 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	1092.00000	546.000000	0.69	0.5141	ns
Storage Temp.	1	1536.00000	1536.00000	1.94	0.1804	ns
PT × ST	2	364.000000	182.000000	0.23	0.7967	ns
Error	18	14232.0000	790.66667			
Total	23	17224.0000				

ns indicates no significant differences

Appendix 4.12: Analysis of variance of germination index of seeds at 6 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	42661.3333	21330.6667	17.81	<.0001	**
Storage Temp.	1	3360.66667	3360.66667	2.81	0.1112	ns
PT × ST	2	2309.33333	1154.66667	0.96	0.4001	ns
Error	18	21556.0000	1197.55556			
Total	23	69887.3333				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.13: Analysis of variance of germination index of seeds at 9 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	6832.0000	3416.0000	11.07	0.0007	**
Storage Temp.	1	228150.000	228150.000	739.2	<.0001	**
PT × ST	2	5776.0000	2888.0000	9.36	0.0016	**
Error	18	5556.0000	308.6667			
Total	23	246314.000				

** indicates highly significant differences at P ≤ 0.0001

Appendix 4.14: Analysis of variance of germination index of seeds at 12 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	50596.0000	25298.0000	58.20	<.0001	**
Storage Temp.	1	152322.667	152322.667	350.4	<.0001	**
PT × ST	2	4585.3333	2292.6667	5.27	0.0157	*
Error	18	7824.0000	434.6667			
Total	23	215328.000				

** indicates highly significant differences at P ≤ 0.0001
* indicates significant differences at P ≤ 0.05

Appendix 4.15: Analysis of variance of germination rate index of seeds at 3 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	4.74345427	2.37172714	0.95	0.4057	ns
Storage Temp.	1	4.49841647	4.49841647	1.80	0.1964	ns
PT × ST	2	0.46576719	0.23288360	0.09	0.9115	ns
Error	18	44.9955782	2.49975435			
Total	23	54.7032162				

ns indicates no significant differences

Appendix 4.16: Analysis of variance of germination rate index of seeds at 6 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	168.456629	84.2283144	17.09	<.0001	**
Storage Temp.	1	13.2582350	13.2582350	2.69	0.1183	ns
PT × ST	2	6.5315495	3.2657748	0.66	0.5277	ns
Error	18	88.7202948	4.9289053			
Total	23	276.966708				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.17: Analysis of variance of germination rate index of seeds at 9 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	23.6774679	11.8387339	8.29	0.0028	**
Storage Temp.	1	713.379142	713.379142	499.4	<.0001	**
PT × ST	2	25.5840438	12.7920219	8.96	0.0020	*
Error	18	25.7122675	1.4284593			
Total	23	788.352922				

** indicates highly significant differences at P ≤ 0.0001
* indicates significant differences at P ≤ 0.05

Appendix 4.18: Analysis of variance of germination rate index of seeds at 12 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	163.126470	81.5632351	45.32	<.0001	**
Storage Temp.	1	703.547755	703.547755	390.9	<.0001	**
PT × ST	2	8.6977778	4.3488889	2.42	0.1176	ns
Error	18	32.3926984	1.7995944			
Total	23	907.764701				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.19: Analysis of variance of coefficient velocity of germination of seeds at 3 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	0.67825422	0.33912711	0.20	0.8218	ns
Storage Temp.	1	1.06217371	1.06217371	0.62	0.4407	ns
PT × ST	2	2.11751646	1.05875823	0.62	0.5493	ns
Error	18	30.7594729	1.70885961			
Total	23	34.6174173				

ns indicates no significant differences

Appendix 4.20: Analysis of variance of coefficient velocity of germination of seeds at 6 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	15.7426692	7.87133459	4.85	0.0207	*
Storage Temp.	1	7.21450251	7.21450251	4.44	0.0493	*
PT × ST	2	9.00339622	4.50169811	2.77	0.0892	ns
Error	18	29.2242078	1.62356710			
Total	23	61.1847757				

* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 4.21: Analysis of variance of coefficient velocity of germination of seeds at 9 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	0.6033889	0.3016944	1.16	0.3346	ns
Storage Temp.	1	193.020635	193.020635	744.9	<.0001	**
PT × ST	2	0.3571349	0.1785674	0.69	0.5148	ns
Error	18	4.6641157	0.2591175			
Total	23	198.645274				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.22: Analysis of variance of coefficient velocity of germination of seeds at 12 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	30.7346304	15.3673152	34.95	<.0001	**
Storage Temp.	1	16.5865985	16.5865985	37.73	<.0001	**
PT × ST	2	12.8357783	6.41788913	14.60	0.0002	**
Error	18	7.91388789	0.43966044			
Total	23	68.0708950				

** indicates highly significant differences at P ≤ 0.0001

Appendix 4.23: Analysis of variance of seedling vigour index of seeds at 3 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	138792.058	69396.0288	0.80	0.4651	ns
Storage Temp.	1	154600.022	154600.022	1.78	0.1988	ns
PT × ST	2	769.2096	384.6048	0.00	0.9956	ns
Error	18	1563189.238	86843.847			
Total	23	1857350.528				

ns indicates no significant differences

Appendix 4.24: Analysis of variance of seedling vigour index of seeds at 6 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	4163242.94	2081621.47	26.05	<.0001	**
Storage Temp.	1	240256.068	240256.068	3.01	0.1000	ns
PT × ST	2	426197.748	213098.874	2.67	0.0967	ns
Error	18	1438252.954	79902.942			
Total	23	6267949.71				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.25: Analysis of variance of seedling vigour index of seeds at 9 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	1318050.70	659025.35	14.87	0.0002	**
Storage Temp.	1	13174387.4	13174387.4	297.3	<.0001	**
PT × ST	2	251610.86	125805.43	2.84	0.0848	ns
Error	18	797609.86	44311.66			
Total	23	15541658.9				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.26: Analysis of variance of seedling vigour index of seeds at 12 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	2470769.80	1235384.90	39.60	<.0001	**
Storage Temp.	1	21833321.4	21833321.4	699.9	<.0001	**
PT × ST	2	197774.29	98887.15	3.17	0.0662	ns
Error	18	561523.02	31195.72			
Total	23	25063388.5				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.27: Analysis of variance of seedling length of seeds at 3 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	1.12093333	0.56046667	0.09	0.9151	ns
Storage Temp.	1	6.74160000	6.74160000	1.07	0.3140	ns
PT × ST	2	0.19960000	0.09980000	0.02	0.9843	ns
Error	18	113.122400	6.2845778			
Total	23	121.184533				

ns indicates no significant differences

Appendix 4.28: Analysis of variance of seedling length of seeds at 6 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	199.200108	99.6000542	21.83	<.0001	**
Storage Temp.	1	28.3185375	28.3185375	6.21	0.0227	*
PT × ST	2	46.0027750	23.0013875	5.04	0.0183	*
Error	18	82.1404750	4.5633597			
Total	23	355.661896				

** indicates highly significant differences at P ≤ 0.0001
* indicates significant differences at P ≤ 0.05

Appendix 4.29: Analysis of variance of seedling length of seeds at 9 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	104.225833	52.1129167	6.00	0.0101	*
Storage Temp.	1	611.050417	611.050417	70.38	<.0001	**
PT × ST	2	22.7194333	11.3597167	1.31	0.2947	ns
Error	18	156.274300	8.6819056			
Total	23	894.269983				

** indicates highly significant differences at P ≤ 0.0001
* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 4.30: Analysis of variance of seedling length of seeds at 12 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	178.746633	89.3733167	57.98	<.0001	**
Storage Temp.	1	920.824817	920.824817	597.4	<.0001	**
PT × ST	2	45.1600333	22.5800167	14.65	0.0002	**
Error	18	27.745700	1.541428			
Total	23	1172.47718				

** indicates highly significant differences at P ≤ 0.0001

Appendix 4.31: Analysis of variance of seedling shoot dry weight of seeds at 3 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	4.96E-6	2.48E-6	0.19	0.8295	ns
Storage Temp.	1	2.66667E-8	2.66667E-8	0.00	0.9646	ns
PT × ST	2	7.05333E-6	3.52667E-6	0.27	0.7675	ns
Error	18	0.00023638	0.00001313			
Total	23	0.00024842				

ns indicates no significant differences

Appendix 4.32: Analysis of variance of seedling shoot dry weight of seeds at 6 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	0.00035188	0.00017594	35.84	<.0001	**
Storage Temp.	1	0.00001980	0.00001980	4.03	0.0599	ns
PT × ST	2	0.00003601	0.00001801	3.67	0.0461	*
Error	18	0.00008837	0.00000491			
Total	23	0.00049607				

** indicates highly significant differences at P ≤ 0.0001
* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 4.33: Analysis of variance of seedling shoot dry weight of seeds at 9 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	0.00017683	0.00008842	11.55	0.0006	**
Storage Temp.	1	0.00066360	0.00066360	86.71	<.0001	**
PT × ST	2	0.00004280	0.00002140	2.80	0.0876	ns
Error	18	0.00013775	0.00000765			
Total	23	0.00102099				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.34: Analysis of variance of seedling shoot dry weight of seeds at 12 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	0.00051661	0.00025831	53.48	<.0001	**
Storage Temp.	1	0.00164011	0.00164011	339.6	<.0001	**
PT × ST	2	0.00016242	0.00008121	16.81	<.0001	**
Error	18	0.00008694	0.00000483			
Total	23	0.00240608				

** indicates highly significant differences at P ≤ 0.0001

Appendix 4.35: Analysis of variance of seedling root dry weight of seeds at 3 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	0.00000650	0.00000325	0.42	0.6607	ns
Storage Temp.	1	0.00000081	0.00000081	0.11	0.7494	ns
PT × ST	2	0.00002386	0.00001193	1.56	0.2380	ns
Error	18	0.00013800	0.00000767			
Total	23	0.00016917				

ns indicates no significant differences

Appendix 4.36: Analysis of variance of seedling root dry weight of seeds at 6 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	0.00008304	0.00004152	10.71	0.0009	**
Storage Temp.	1	0.00008664	0.00008664	22.35	0.0002	**
PT × ST	2	0.00003163	0.00001582	4.08	0.0346	*
Error	18	0.00006978	0.00000388			
Total	23	0.00027109				

** indicates highly significant differences at P ≤ 0.0001
* indicates significant differences at P ≤ 0.05

Appendix 4.37: Analysis of variance of seedling root dry weight of seeds at 9 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	0.00011482	0.00005741	16.44	<.0001	**
Storage Temp.	1	0.00036974	0.00036974	105.9	<.0001	**
PT × ST	2	0.00001783	0.00000892	2.55	0.1056	ns
Error	18	0.00006285	0.00000349			
Total	23	0.00056524				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.38: Analysis of variance of seedling root dry weight of seeds at 12 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	0.00027130	0.00013565	29.54	<.0001	**
Storage Temp.	1	0.00057820	0.00057820	125.9	<.0001	**
PT × ST	2	0.00005214	0.00002607	5.68	0.0123	*
Error	18	0.00008267	0.00000459			
Total	23	0.00098432				

** indicates highly significant differences at P ≤ 0.0001
* indicates significant differences at P ≤ 0.05

Appendix 4.39: Analysis of variance of catalase activity of seeds at 3 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	0.08448711	0.04224356	0.05	0.9526	ns
Storage Temp.	1	0.71011417	0.71011417	0.82	0.3831	ns
PT × ST	2	0.20614855	0.10307428	0.12	0.8889	ns
Error	12	10.3969839	0.86641532			
Total	17	11.3977337				

ns indicates no significant differences

Appendix 4.40: Analysis of variance of catalase activity of seeds at 6 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	9.53352562	4.76676281	11.77	0.0015	**
Storage Temp.	1	4.83646468	4.83646468	11.94	0.0048	**
PT × ST	2	1.60525511	0.80262756	1.98	0.1805	ns
Error	12	4.86138837	0.40511570			
Total	17	20.8366338				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.41: Analysis of variance of catalase activity of seeds at 9 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	1.72438194	0.86219097	2.08	0.1681	ns
Storage Temp.	1	17.0668189	17.0668189	41.10	<.0001	**
PT × ST	2	1.06707221	0.53353611	1.28	0.3122	ns
Error	12	4.98304981	0.41525415			
Total	17	24.8413228				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.42: Analysis of variance of catalase activity of seeds at 12 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	3.04238087	1.52119044	4.73	0.0305	*
Storage Temp.	1	11.6406342	11.6406342	36.21	<.0001	**
PT × ST	2	0.64463666	0.32231833	1.00	0.3957	ns
Error	12	3.85768149	0.32147346			
Total	17	19.1853332				

** indicates highly significant differences at P ≤ 0.0001
* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 4.43: Analysis of variance of guaiacol peroxidase of seeds at 3 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	85.9628794	42.9814397	4.53	0.0342	*
Storage Temp.	1	473.314398	473.314398	49.88	<.0001	**
PT × ST	2	20.0384137	10.0192069	1.06	0.3781	ns
Error	12	113.868102	9.4890085			
Total	17	693.183793				

** indicates highly significant differences at P ≤ 0.0001
* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 4.44: Analysis of variance of guaiacol peroxidase of seeds at 6 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	58.0626879	29.0313440	2.82	0.0990	ns
Storage Temp.	1	285.289606	285.289606	27.73	0.0002	**
PT × ST	2	9.3096027	4.6548014	0.45	0.6465	ns
Error	12	123.453181 7	10.2877651			
Total	17	476.115079				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.45: Analysis of variance guaiacol peroxidase of seeds at 9 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	4.9645554	2.4822777	0.08	0.9212	ns
Storage Temp.	1	401.014976	401.014976	13.35	0.0033	**
PT × ST	2	72.0336961	36.0168481	1.20	0.3352	ns
Error	12	360.4952161	30.0412680			
Total	17	838.508444				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.46: Analysis of variance of guaiacol peroxidase of seeds at 12 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	210.255069	105.127535	13.93	0.0007	**
Storage Temp.	1	287.836822	287.836822	38.15	<.0001	**
PT × ST	2	62.4530189	31.2265095	4.14	0.0430	*
Error	12	90.5469246	7.5455770			
Total	17	651.091836				

** indicates highly significant differences at P ≤ 0.0001
* indicates significant differences at P ≤ 0.05

Appendix 4.47: Analysis of variance of superoxide dismutase of seeds at 3 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	0.91170992	0.45585496	1.31	0.2950	ns
Storage Temp.	1	0.09777993	0.09777993	0.28	0.6029	ns
PT × ST	2	0.02942474	0.01471237	0.04	0.9588	ns
Error	18	6.27653411	0.34869634			
Total	23	7.31544870				

ns indicates no significant differences

Appendix 4.48: Analysis of variance of superoxide dismutase of seeds at 6 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	2.60392391	1.30196196	4.07	0.0348	*
Storage Temp.	1	2.59609751	2.59609751	8.11	0.0107	*
PT × ST	2	3.71046874	1.85523437	5.80	0.0114	*
Error	18	5.75979522	0.31998862			
Total	23	14.6702854				

* indicates significant differences at P ≤ 0.05

Appendix 4.49: Analysis of variance of superoxide dismutase of seeds at 9 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	4.73332038	2.36666019	13.52	0.0003	**
Storage Temp.	1	4.52060726	4.52060726	25.83	<.0001	**
PT × ST	2	0.38722920	0.19361460	1.11	0.3522	ns
Error	18	3.15010627	0.17500590			
Total	23	12.7912631				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.50: Analysis of variance of superoxide dismutase of seeds at 12 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	1.28388891	0.64194445	4.09	0.0343	*
Storage Temp.	1	8.28515345	8.28515345	52.82	<.0001	**
PT × ST	2	0.03272764	0.01636382	0.10	0.9015	ns
Error	18	2.82347118	0.15685951			
Total	23	12.4252412				

** indicates highly significant differences at P ≤ 0.0001
* indicates significant differences at P ≤ 0.05
ns indicates no significant differences

Appendix 4.51: Analysis of variance of malondialdehyde of seeds at 3 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	1.43073244	0.71536622	10.93	0.0008	**
Storage Temp.	1	0.00006468	0.00006468	0.00	0.9753	ns
PT × ST	2	0.03362892	0.01681446	0.26	0.7762	ns
Error	18	1.17809275	0.06544960			
Total	23	2.64251880				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.52: Analysis of variance of malondialdehyde of seeds at 6 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	0.38250156	0.19125078	0.79	0.4702	ns
Storage Temp.	1	4.38768913	4.38768913	18.06	0.0005	**
PT × ST	2	0.29774476	0.14887238	0.61	0.5528	ns
Error	18	4.37368861	0.24298270			
Total	23	9.44162407				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.53: Analysis of variance of malondialdehyde of seeds at 9 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	0.32256192	0.16128096	1.63	0.2235	ns
Storage Temp.	1	3.39182091	3.39182091	34.28	<.0001	**
PT × ST	2	0.01464324	0.00732162	0.07	0.9290	ns
Error	18	1.78096870	0.09894271			
Total	23	5.50999477				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 4.54: Analysis of variance of malondialdehyde of seeds at 12 months after storage

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Packaging Type	2	1.88555692	0.94277846	6.22	0.0089	**
Storage Temp.	1	10.8041737	10.8041737	71.24	<.0001	**
PT × ST	2	0.26650141	0.13325071	0.88	0.4324	ns
Error	18	2.72974206	0.15165234			
Total	23	15.6859741				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 5.1: Analysis of variance of final germination percentage of trial, with concentration of 0 to 100 mM of hydrogen peroxide priming concentration.

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Priming Conc.	9	1001.6934	111.2992	8.09	<.0001	**
Block	3	12.5690	4.1896	0.30	0.732	ns
Error	27	371.1418	13.7459			
Total	39	1035.4042				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 5.2: Analysis of variance of final germination percentage of seeds treated with different hydrogen peroxide priming concentration between 0 mM to 20 mM

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Priming Conc.	9	1851.6000	3.733333	5.50	0.0003	**
Block	3	33.200000	11.066667	0.30	0.8282	ns
Error	27	1010.80000	37.437037			
Total	39	2895.60000				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 5.3: Analysis of variance of mean germination time of seeds treated with different hydrogen peroxide priming concentration between 0 mM to 20 mM

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Priming Conc.	9	1.91940969	0.21326774	6.76	<.0001	**
Block	3	0.05389768	0.01796589	0.57	0.6401	ns
Error	27	0.85221138	0.03156338			
Total	39	2.82551875				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 5.4: Analysis of variance of germination index of seeds treated with different hydrogen peroxide priming concentration between 0 mM to 20 mM

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Priming Conc.	9	47960.0000	5328.88889	9.17	<.0001	**
Block	3	707.2000	235.73333	0.41	0.7500	ns
Error	27	15684.8000	580.91852			
Total	39	64352.0000				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 5.5: Analysis of variance of germination rate index of seeds treated with different hydrogen peroxide priming concentration between 0 mM to 20 mM

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Priming Conc.	9	181.944454	20.2160504	8.90	<.0001	**
Block	3	2.0515011	0.6838337	0.30	0.8243	ns
Error	27	61.3250068	2.2712965			
Total	39	245.320962				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 5.6: Analysis of variance of germination rate of seeds treated with different hydrogen peroxide priming concentration between 0 mM to 20 mM

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Priming Conc.	9	151.265816	16.8073129	7.93	<.0001	**
Block	3	3.0056122	1.0018707	0.47	0.7040	ns
Error	27	57.2545918	2.1205404			
Total	39	211.526020				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 5.7: Analysis of variance of coefficient velocity of germination of seeds treated with different hydrogen peroxide priming concentration between 0 mM to 20 mM

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Priming Conc.	9	54.1941015	6.02156684	7.49	<.0001	**
Block	3	1.31147589	0.43715863	0.54	0.6564	ns
Error	27	21.6992024	0.80367416			
Total	39	77.2047798				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 5.8: Analysis of variance of seedling length of seeds treated with different hydrogen peroxide priming concentration between 0 mM to 20 mM

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Priming Conc.	9	148.406840	16.4896489	2.56	0.0282	**
Block	3	9.4880400	3.1626800	0.49	0.6910	ns
Error	27	173.684760	6.4327689			
Total	39	331.579640				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences.

Appendix 5.9: Analysis of variance of seedling vigour index of seeds treated with different hydrogen peroxide priming concentration between 0 mM to 20 mM

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Priming Conc.	9	3394341.74	377149.082	8.09	<.0001	**
Block	3	22876.635	7625.545	0.16	0.9200	ns
Error	27	1258948.63	46627.727			
Total	39	4676167.00				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences.

Appendix 5.10: Analysis of variance of seedling root dry weight of seeds treated with different hydrogen peroxide priming concentration between 0 mM to 20 mM

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Priming Conc.	9	0.00005496	0.00000611	0.96	0.4914	ns
Block	3	0.00002718	0.00000906	1.43	0.2566	ns
Error	27	0.00017141	0.00000635			
Total	39	0.00025356				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 5.11: Analysis of variance of seedling shoot dry weight of seeds treated with different hydrogen peroxide priming concentration between 0 mM to 20 mM

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Priming Conc.	9	0.00027645	0.00003072	3.73	0.0038	**
Block	3	0.00005978	0.00001993	2.42	0.0882	ns
Error	27	0.00022262	0.00000825			
Total	39	0.00055886				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 5.12: Analysis of variance of guaiacol peroxidase activity of seeds treated with different hydrogen peroxide priming concentration between 0 mM to 20 mM

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Priming Conc.	9	111.002649	12.3336277	31.12	<.0001	**
Block	3	0.8872095	0.2957365	0.75	0.5340	ns
Error	27	10.7019158	0.3963673			
Total	39	122.591775				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 5.13: Analysis of variance of superoxide dismutase of seeds treated with different hydrogen peroxide priming concentration between 0 mM to 20 mM

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Priming Conc.	9	16.0867179	1.78741310	13.19	<.0001	**
Block	3	0.51311573	0.17103858	1.26	0.3071	ns
Error	27	3.65851208	0.13550045			
Total	39	20.2583457				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 5.14: Analysis of variance of catalase activity of seeds treated with different hydrogen peroxide priming concentration between 0 mM to 20 mM

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Priming Conc.	9	35.0881098	3.89867886	12.11	<.0001	**
Block	3	0.94496722	0.31498907	0.98	0.4174	ns
Error	27	8.69099902	0.32188885			
Total	39	44.7240760				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 5.15: Analysis of variance of malondialdehyde of seeds treated with different hydrogen peroxide priming concentration between 0 mM to 20 mM

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Priming Conc.	9	61.4532752	6.82814169	20.50	<.0001	**
Block	3	2.93877330	0.97959110	2.94	0.0510	ns
Error	27	8.99335736	0.33308731			
Total	39	73.3854059				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

Appendix 5.16: Analysis of variance of electrical conductivity of seed Leachate of seeds treated with different hydrogen peroxide priming concentration between 0 mM to 20 mM

Source of Variation	DF	SS	MS	F	Pr > F	Notes
Priming Conc.	9	8375.43965	930.604405	7.10	0.0002	**
Block	2	179.177307	89.588653	0.68	0.5175	ns
Error	18	2359.54332	131.08574			
Total	29	10914.1603				

** indicates highly significant differences at P ≤ 0.0001
ns indicates no significant differences

BIODATA OF STUDENT

Sharif Azmi bin Abdurahman was born on the December 9th, 1989, in a small village of Kunak, Sabah, Malaysia. He received his primary education at Mostyn Middle School and secondary education at Sekolah Menengah Kebangsaan Kunak in Sabah. He then furthered his study in Diploma of Planting Industry Management and Bachelor of Science (Hons) Plantation Technology and Management from Universiti Teknologi MARA. After graduated, he worked for Universiti Teknologi MARA in 2013 before joining Universiti Malaysia Sabah as an academic staff in 2014. In 2019, he was awarded with scholarship under Ministry of Higher Education for Doctor of Philosophy degree in Universiti Putra Malaysia. His passion towards seed has encouraged him to further his studies in seed science and technology.