AGRONOMIC CHARACTERISTICS AND POLLEN MORPHOLOGY AMONG TROPICAL SWEET CORN HYBRID VARIETIES

By

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ABSTRACT

AGRONOMIC CHARACTERISTICS AND POLLEN MORPHOLOGY AMONG TROPICAL SWEET CORN HYBRID VARIETIES

Yong Yan Shee

The development of local sweet corn hybrid varieties from the utilization of imported genetic materials would help to reduce reliance on imported seeds. In this study, five imported tropical sweet corn hybrid varieties were planted with Randomized Complete Block Design (RCBD) in Kampar, Perak to investigate their agronomic characteristics. The measured traits were analyzed using analysis of variance (ANOVA), followed by interpretation of their broad-sense heritability and phenotypic correlations. The results revealed that hybrid varieties GreenEagle 1602 Supersweet and Leckat 592 Sugar Rich with superior performance in pre- and post-harvest traits, relatively could be short-listed for the development of inbred lines. Broad-sense heritability (h^2_B) estimates revealed from the variance components method were found high for total suspended solid (99.32%), ear height (98.75%), kernel length (98.53%), number of husks per ear (97.89%) and plant height (97.08%). Among the traits that were highly correlated to dehusked ear weight were dehusked dried ear weight (0.81), dehusked ear length (0.59), dehusked ear diameter (0.57), and number of husks per ear (0.54). For the selection purpose, the evaluated hybrid varieties with the

highest number of husks per ear were suggested as the genetic material for yield improvement. Therefore, Leckat 592 Sugar Rich could be potential genetic resource for local inbred line development with the highest number of husks per ear (12.67 ± 0.19). On the other hand, the pollen grains from five different hybrid varieties were observed under a light microscope and scanning electron microscope (SEM). The morphology of pollen observed was circular and monoporate, and their size is approximately 100 μm, which their sizes and structure were found similar from both views. Collectively, these agronomic characteristics and pollen morphology could be acting as essential information for future study in optimizing production yield and assisting material for the identification of pollen development stages.

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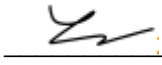
DECLARATION

I am hereby to declare that this final year project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.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LIST OF ABBREVIATIONS

Ag Park B	Agricultural Park B
ANOVA	Analysis of variance
DDEW	Dehusked dried ear weight
DED	Dehusked ear diameter
DEL	Dehusked ear length
DEW	Dehusked ear weight
DEY	Dehusked ear yield
DF	Degree of freedom
DMNRT	Duncan's multiple range test
EFB	Empty fruit bunch
EH	Ear height
F_1	First generation
KL	Kernel length
NHE	Number of husks per ear
NKR	Number of kernels per row
NKRE	Number of kernel rows per ear
NL	Number of leaves

NPK	Nitrogen, Phosphorus, Potassium
PH	Plant height
RCBD	Randomized complete block design
rpm	Revolutions per minute
SAS	Statistical analysis system
SD	Stem diameter
SEM	Scanning electron microscope
TL	Tassel length
TSS	Total soluble solids

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Corn which could be known as maize is the leading cereal crop in the world with various corn varieties, including popcorn, flour corn, dent corn, flint corn, sweet corn, striped maize, and Amylomaize (Revilla, Anibas and Tracy, 2021). It is one of the important food sources as fresh or processed vegetables that aimed in nutritional security, hence, they have been cultivated worldwide by farmers that have diverged choices of selection. According to Food and Agriculture Organization (FAO), maize including sweet corn was found as the globally top sixth produced commodities with the record of total 1,161 million tons produced worldwide from year 2010 to year 2020 (FAOSTAT, 2020). Among these varieties, specialty corn production was most favored by corn growers because they have high market returns with more employment opportunities offered to urban generations (Swapna, Jadesha and Mahadevu, 2020). Sweet corn is one of the specialty corns that distinguished from other corn varieties due to its high nutritional value that promote human health. It is also known as *Zea mays* L. *saccharata.* which is from grass family Poaceae that formerly called Gramineae (Revilla, Anibas and Tracy, 2021). Originally, sweet corn is found in the United States of America (USA) and gaining its popularity due to its high kernel sweetness that possess high sugar contents (Chhabra, et al., 2022).

Sweet corn produced a much-sweetened taste when consumed fresh which is around six-times higher sweetness that other field corn due to the increasing number of recessive alleles in the corn endosperm starch synthesis pathway (Revilla, Anibas and Tracy, 2021). Therefore, breeders were select the recessive shrunken2 (*sh2*) gene that is located on the chromosome 3L at bin location 3.09 to develop and commercialize the modern sweet corn (Chhabra, et al., 2022). The modern sweet corn with an altered endosperm starch synthesis pathway would disrupt the polysaccharide composition to produce higher sugar content with lowered starch content synthesized, hence is more suitable for human consumption (Tracy, Shuler and Dodson-Swenson, 2020).

Sweet corn cultivation started to gain popularity from the introduction of the variety Chinta into local market in the early sixties (Rosali, et al., 2019). From that point forward, farmers in Malaysia started to show their interest in selecting sweet corn due it its low production cost and shortened cultivation period (Rosali, et al., 2019). This was further proved by the rapid emergence of sweet corn cultivation areas in Malaysia from 2003 (6,591 hectares) to 2018 (11,713 hectares) (Kasron, et al., 2023). The trend of sweet corn cultivation was further pushed the production of sweet corn in Malaysia from 31,907 tons to 84,170 tons, which increased by approximately 62% in the total production of sweet corn in Malaysia (DOSM, 2021). The sweet corn also results in high self-sufficiency ratio (SSR) of around 106% and high economic returns as reported by DOSM (2021) and Rosali, et al. (2019). Therefore, sweet corn is an essential

crop as it plays an important row in human consumption in Malaysia. Even though the potential for sweet corn to be the main nutritional source for humans is yet to be promoted natively, they are proven to have good levels of valuable vitamins that could be protecting the human nervous and digesting system.

In Malaysia, sweet corn cultivation utilizes both local varieties and imported hybrid varieties. Local open-pollinated composite varieties developed by the Malaysian Agricultural Research and Development Institute (MARDI) including Bakti-1, Mas Madu, Mains Madu, Thai Supersweet, Hibridmas, and Super Sweet Red (Saat, et al., 2020). The yields obtained from those local composite varieties are remained between ranging of moderate to low quality regarding their sweetness or growth conditions which could not cope with the demand of sweet corn in Malaysia. According to Kashiani, et al. (2014), the genetic alteration for lacking quality-inbred lines could be the cause for raising potential challenges including the germination, emergence, tolerance, and fruiting quality for the local sweet corn hybrids. In opposed to imported tropical sweet corn hybrid varieties, they could promise high quality fruits and high yield production in Malaysia's habitat quality which led to Malaysia's heavy reliance on 95% of imported sweet corn hybrid seeds from countries such as Taiwan, Thailand, and China (Nazmi, et al., 2021; Ng and Tong, 2015). The heavily reliance of imported sweet corn hybrid seeds also lead to high expenses which Hamid (2018) reported that the high expenses of food import bills in Malaysia had cost approximately RM 45.4 billion in the year 2018 which the imported hybrid crop seeds played one of the major roles in the expenses.

There is a compelling need to introduce the local breeding program to develop the high-quality inbred lines from genetic sources of imported hybrids to crossbreed with local inbred lines and form superior local hybrid varieties (Hishamuddin, 2023). The local breeding program is a process that aiming to develop superior inbred lines and subsequently high yielding sweet corn hybrid varieties for Malaysia (Hishamuddin, 2023). In addition, the local plant breeding activities still mainly relied on government agencies due to institutional weakness that lack of researchers in looking for the elite imported populations and conduct agronomic characteristics comparison (Ng and Tong, 2015). Primary steps in plant breeding program including evaluation on agronomic performance and analysis on variation in sweet corn pollen in different foreign sweet corn hybrid varieties are essential for identifying their eligibility for conventional breeding or double haploid program. Therefore, a study of outperformed imported tropical sweet corn hybrid varieties is essential for selecting the good qualities of imported sweet corn genetic materials. The selected foreign materials could be potentially utilized with local open-pollinated composite varieties to greatly improve local hybrid varieties that thrive in Malaysia's environmental conditions and pose high-yielding and consuming quality with lowered seed prices. As consequence, study of agronomic performance of sweet corn hybrids is essential for elite source population selection. Nevertheless, Swapna, Jadesha and Mahadevu (2020) mentioned that sweet corn is harvested during the milk stage of endosperm development. Therefore, harvesting the sweet corn in optimal timing should be a concern during evaluation for their performance. Overall, development of a

long, sweet corn breeding program would be essential for agriculture development in Malaysia. This practice could greatly reduce the burden on the cost of production from Malaysia's farming communities that heavily relied on imported tropical sweet corn hybrid planting materials due to poor development of local varieties (Kashiani, et al., 2014).

On top of that, pollination is the most critical period for sweet corn endosperm development and could directly affect the corn's quality and yield. Sweet corn pollen is a plant's male gamete which derived from anthers. It could be fertilized with potential female ovule in ear to form the sweet corn kernel. Therefore, pollen grain is crucial for promising the sweet corn yield (Choe, Ko and Williams, 2021). Moreover, pollen grains as the crucial component in successful pollination could play another important role as one of the significant tools for solutions to taxonomic challenges of sweet corn identification regarding their family, generic, and specific level placement (Gabr, 2018). Besides, the study of pollen morphology and their development characteristics are not yet investigated thoroughly (Zhang, et al., 2013). Therefore, the study of sweet corn pollen morphology could be essential for getting more useful information such as their development variations among varieties and essential structure for pollination that could be incorporate with the agronomic performance of sweet corn in order to select the most suitable sweet corn inbred line for developing the superior local hybrid varieties.

In this study, the comparison of agronomic performance for five different foreign tropical sweet corn hybrid varieties, including Agroniche Big Bicorn 317, CropPower Asia Manis SS-932, GreenEagle Jagung Manis Asia Best 1602, Green World Genetics Akik Sc-422 and Leckat Sugar Rich 592 was conducted to select the outperformed variety that poses quality traits that could be developed into superior local inbred lines. The pollen morphology study, in contrary was conducted using light microscope and scanning electron microscope (SEM) approaches to compare the pollen's morphology between the sweet corn varieties and acted as additional information for further studies of pollen development stages.

1.2 Objectives

The objectives of this study were:

1. To evaluate the agronomic characteristics of five tropical sweet corn hybrids planted in Kampar, Perak.
2. To estimate broad-sense heritability of the characteristics measured.
3. To study morphological variations of sweet corn pollen from those varieties using scanning electron microscopy (SEM).

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction of Sweet Corn

2.1.1 History of Maize

Maize that could be known as *Zea mays* ssp. *Mays* scientifically was belonging to a tribe named Maydae from family Poaceae (Yanga, et al., 2013). The origin of maize could be traced back to naming of it as the term 'maize' was assumed to be derived from the word 'mahiz' of Taino language from Caribbean islands, after that, the word 'mahiz' became 'maiz' in Spanish (Oxford University Press, 2015). As consequence, maize was believed to be originated in Mexico and Central America. However, the evolution for maize is still a great scientific challenge for both biologists and archaeologists (Hossain, et al., 2016). There were four hypotheses had been stated simultaneously to prove the origin of maize, among them included Tripartite hypothesis from year 1939, Catastrophic sexual transmutation theory from year 1983, *Tripsacum-Zea diploperennis* hypothesis from year 1995 and lastly Teosinte hypothesis from year 1939 (Hossain, et al., 2016). Recently, Balsa teosinte (*Zea mays* spp. *Parviglumis*) was accepted as the progenitor of maize although they had superior morphological variation persisted between each other (Pruitt, 2016). All the species for teosinte was proved to have ability crossing with maize and majority of the traits presented by maize nowadays was believed to be donated by

teosinte (Wang, et al., 2014). Furthermore, maize was domesticated by people about 9000 years ago and they were improved by crossbreeding for desired traits (Hossain, et al., 2016). Originally, maize plants with transposable element insertion that could tolerate long-day environments had been selected by mankind for the formation of tropical maize plants nowadays (Yanga, et al., 2013). The long-term selection for maize had developed them the ability to adapt in diverse environments that promoted their widespread and promising crop yield. As a result, annual stapled modern maize production serves as the livestock feeds and for human consumption (Prasanna, 2014).

2.1.2 Classification of Sweet Corn

Maize that is globally top sixth produced crops served as the majority source of food and nutrition content for the developing countries (FAO, 2022). Among the various types of maize including flint corn, popcorn, dent corn, sweet corn and more. Recently, sweet corn has been gaining popularity in both fresh market and food processing industry (Mitewty, et al., 2019). The popularity and demand of sweet corn has been substantially increased to US$ 1541.72 million and there is around US$ 1475 million worth for import and export during 2020 (Chhabra, et al., 2022). However, limited information for the cloning of recessive shrunken2 (*sh2*) gene is available due to sweet corn breeding is limited to few developed countries (Markovic, et al., 2020). As consequence, the urge for developing countries to have own sweet corn inbred lines is essential for the sufficient supplying of market demand.

2.1.3 Genetics of Sweet Corn

Sweet corn is differed from other maize types regarding of its six-fold sweeter of the kernel taste, the high amount of sugar content identified in the kernel possess multivitamins to ensure nutritional security (Singh, Langyan and Yadava, 2014). The modification of kernel sweetness was attributed to the genetically transformation of starch biosynthesis pathway that reduced sugar content synthesizing into starch. It worked by one or more recessive mutations that blocking conversion of sugar content to starch during the sweet corn kernel formation therefore resulted in kernels that are high in sugar content while low in starch content. Four separated gene mutation could contribute to sweetness in corn including normal sugary (*su*), sugary enhanced (*se*), brittle1 (*bt*) and shrunken2 (*sh2*). Among them, most of the sweet corn modification was conducted using recessive shrunken2 (*sh2*) gene that located on chromosome 3L at bin location of 3.09 (Chhabra, et al., 2022). The recessive genes were initially identified as a defect trait but later artificially selected by humankind for their uniqueness.

2.2 Botany and Morphology of Sweet Corn

2.2.1 Structure of Sweet Corn

Sweet corn is a tall plant with fibrous rooting system. As an erect annual grass, sweet corn was typically cultured in well-prepared seedbed with 25 to 75 cm apart that leads to favorable positioning of growing point (Mitewty, et al., 2020). Sweet corn reaches 1 to 2 m in height and possesses a main culm with nodes

and internodes developed within. The formation of one to two lateral branches in the lower leaf axils are typically known as tillers and its formation relies on the genetic coding and plant population density of sweet corn (Shahriari, 2013). The leaf blades of sweet corn plant possess parallel veins with prominent midrib, and they are arranged in two vertical rows with opposite positioning on sides of the stalk. The variation of leaves, size and orientation of sweet corn leaves is depended on varieties, with tropical hybrids producing up to 18 leaves (Chhabra, et al., 2022). Besides, sweet corn upper leaf blade is equipped with hairy characteristics whereas the lower surface is hairless.

Sweet corn is a monocotyledon and monoecious plant that develops separated male and female flowers on each individual. This trait is known as imperfect flowers while the staminate flower and pistallate flower of sweet corn are generally known as tassel and ear, respectively (Tadros, Omari and Turk, 2019). The leaves that initially covered the ear on axillary shoots are known as husks which having diverse appearance from leaf blades on plant stalk. The purpose of husks development is to surround the ear while they are in maturation and protecting them from external circumstances (Tamthong, Kraisuwan and Piromthamsiri, 2022). Inside the ear consists of cob that develops averagely 4 to 30 rows of florets with each contains a single ovule (Hommood, et al., 2020). The style from the florets will elongate towards tip of the cob and therefore forming long strands of whitish to greenish shorthaired tissues known as silks. In some situations, silk tissues may turn brown from mechanical damage due to oxidation of phenolic compounds (Ortez, et al., 2022).

On the other hand, tassels of sweet corn are prominent branch structures located at the end of tell erect stem consisting of a central spike and variable lateral branches. The tassels will be forming anthers that colored in yellow, purple or red (Duangpapeng, et al., 2018). Each anther consists numerous pollen grains that could be fertilizing the ovule and forming kernels (Adiaha, 2017). The kernel often presented in distinct coloration that vary between varieties. The pericarp of the kernel can be colorless, brown, or yellow. The brown aleurone could be resulted from the presence of anthocyanins, white could be due to the lack of anthocyanins and carotenoids or yellow could be resulted from presence of carotenoids (Germen, et al., 2011). As consequence, the unique physiochemical properties of sweet corn produced wide range of sweet corn varieties.

2.2.2 Physiology of Sweet Corn

Sweet corn plants came in different varieties with various characteristics that depend on their origin, but they have similar phases of development from planting to maturity. The life cycle of sweet corn could be distributed into four distinct phases including the leaf-growth phase, the grain-filling period, the flowering period and lastly the period of grain dry down (Tracy, Shuler and Swenson, 2020). According to Sharma, et al. (2015), sweet corn seeds are susceptible to diseases and insects, therefore obtaining certified chemical treated seeds were crucial for success cultivation. During the leaf-growth phase, sweet corn seedlings could be emerged in as few as three days from warm and

moisture soil conditions. The leaves would appear above ground upon the seed emergence; however, growing point of the sweet corn is still below ground until forth to fifth leaves emergence. During the four-leaf stage with four visible leaves, the plant will go through a crucial event known as ‘short growth crisis’ (Fletcher, Moot and Stone, 2002). The sweet corn seedlings in this stage would have a metabolic transition from heterotrophic to autotropic growth that the seedlings total relied on sunlight for nutrient supply (Ceccoli, et al., 2014). Sweet corn seedlings at this stage would be susceptible to particular unfavorable conditions including soil temperature, soil compaction and soil moisture. As consequence, the effects of seedlings management would be magnified and became essential for success.

About reaching the eight- to ten- leaf stage, sweet corn would be going through process of tassel initiation (Ciampitti, Elmore and Lauer, 2016). The leaves continued to expand associated with topmost leaf emergence from whorl. Subsequently, tassels would be evident from fully development of topmost leaf. As for the ear development, sweet corn plants were commonly known for only one ear developed for each plant, in some cases, multiple ear development might be occurred during this stage (Ortez, et al., 2022). Additionally, ear was developed from topmost axillary meristem that initiated on same day as the tassel. However, axillary meristem located five to seven leaves below the tassel would results in late transaction from axillary meristem to ear formation due to delay of plastochrons for around 10 days after the tassel initiation (Donald and Chantal, 1999). As reported by Pinjari and Chavan (2019), this delaying process would help to time the pollen and ovule maturation for perfect fertilization. The

transition of excess ear from axillary buds would be progress towards lower nodes therefore the topmost ear would be the primary ear for kernel production (Ortez, et al., 2022). The ear in lower nodes would usually abort before silking process but favorable environmental conditions such as presence of high solar irradiance, nutrient sufficiency and relaxed growing point would prevent their abortion (Shao, et al., 2021).

Subsequently, upon the maturation of the imperfect flowers, sweet corn would be attaining its peak height and matured pollen would be shed from tassels (Sirih, et al., 2021). The matured pollen would be trapped from silks emerging from earshoot. Pollen production from the florets was abundant with approximately 10 million pollen grains shed per day per plant, therefore, limitation of insufficient pollen was seldom (Sirih, et al., 2021). The emerged silks from sweet corn earshoot typically fertilized within eight days as the pollen grains would need to develop pollen tubes all the way down to the entire silk length before fertilization (Sulewska, et al., 2014). However, kernel abortion and/or failure in florets fertilization may occurred from stress factors such as nutrient deficiency, excessive heat, pest or highly competitive plant density. This incident would result in yield loss or incomplete seed set with topmost cob lacking kernel development (Dai, Ma and Song, 2021). Flowering period of sweet corn is vulnerable for the plant, as they required peaked demand of resources. The external factors such as heat and water stress would be promoting early flowering which resulting in high risk of floral fertilization failure (Waqas, et al., 2021).

After the silks from topmost ear were successfully fertilized from two to three weeks, rapid filling of grain would take place within the ear. The grain would be growing in a rate of approximately 4% of final yield per day during this period (Djaman, et al., 2022). Sweet corn plant in this stage was highly sensitive to prevailing temperature due to dry matter accumulation in the ear. Stress induced in this period would result in premature leaf death on the plant. Grain maturity could be traced according to presence of milky fluid from the kernels (Randby, et al., 2019). This process involved pinching random kernels from desired ears for the occurrence of semi-transparent milky fluid. An indication from dried brownish silks and milky kernel fluid would be a hint to matured sweet corn. Sweet corn in these stages was usually reaching grain moisture of 40 to 45%. Prolonged unharvested sweet corn would result in continuous accumulation of dry matter that resulted in high starch content (Costa, et al., 2018). Unharvested ear would result in rapid loss of moisture content which called as grain dry down, but this process could be prolonged from high air humidity (Costa, et al., 2018). As consequence, timing of harvesting sweet corn is crucial for preservation of the total soluble solid content.

2.2.3 Economic Value of Sweet Corn in Malaysia

In Malaysia, the popularity and demand of local sweet corn production is increasing recent years due to their high nutritional values and sweetened aroma (Statista, 2022). Based on the record from Malaysia Department of Agriculture (2022), the native sweet corn production from year 2021 were concluded at a

value of 73 thousand megaton (MT). Besides, the total cropping area for sweet corn production was recorded at value of 10 thousand hectare. The top three areas in Malaysia having highest sweet corn production were Perak, Johor, and Sarawak with 23 thousand MT, 12 thousand MT and 9 thousand MT, respectively (Kasron, et al., 2023). Recently, local sweet corn production was increasing to fulfill the market demand. In agreement, sweet corn importation was decreased from 5,013 MT in year 2016 to 3,288 MT in year 2020 to reduce import reliance for the according crop (Kasron, et al., 2023). For instance, Singapore as the main importer for fresh local sweet corn from Malaysia had contributed to increased local sweet corn exporting value from 5,670 MT in year 2016 to 6,882 MT in year 2020 (Malaysia Department of Agriculture, 2022). These recorded data was solid proof to the economic importance of sweet corn production to Malaysia and even worldwide.

As sweet corn gaining popularity in Malaysia, it had penetrated local markets with various options including yellow corn, pearl corn, purple corn, milk corn, sugar-rich bicorn and more (Kasron, et al., 2023). Among them, yellow corn and pearl corn were the famous sweet corn that contributed to multipurpose consumption choices whereas others were less known by the consumers. Various studies have been conducted locally for their benefits, nutritional composition, recipes, and consumer's willingness to consume them. However, sweet corn market pricing in Malaysia was still a challenge for the farmers and researchers. This is because the sweet corn production in Malaysia may be varied from multiple factors to single climate change that affecting the weather (Jamaludin, and Zeid, 2015). For example, sweet corn variety may not be

mainly produced from farmers due to interference from pest invasion, profit returning and area crop demand. Besides, the agriculture machinery utilization in Malaysia for sweet corn production is considered lower as compared to other local crops (Isaak, et al., 2020). The unstable production margin had contributed to the uncertainty of local sweet corn pricing which affecting consumer's willingness for sweet corn consumption.

This raised the attention from Malaysia Department of Agriculture and Food Security by seeking economic importance of sweet corn and problems encountered. Federal Agricultural Marketing Authority (FAMA) had developed sweet corn grading index with grade premium, grade 1 and grade 2 (FAMA, 2022). The grading specifications were mainly based on their sizes and appearance whereas other traits such as maturity, freshness and cleanliness should be standardized for all grades. Sweet corn in Malaysia market was classified into large (L), medium (M) and small (S) sizes with respective grade from premium to grade 2. Premium graded sweet corn has the length of > 19.0 cm and diameter of > 5.0 cm; grade 1 sweet corn was classified with sweet corn length between 17.1 cm to 19.0 cm with diameter between 4.1 cm to 5.0 cm; grade 2 sweet corn was sized with length from 15.0 cm to 17.0 cm and diameter from 3.5 cm to 4.0 cm (FAMA, 2022). The sweet corn with lower size specifications than grade 2 standard would results in tremendous drop of their market value. Therefore, size is one of the major quality parameters that contributed to consumers' willingness or preference in purchasing the crop (Kaur, 2017).

2.3 Sweet Corn Breeding

2.3.1 Plant Breeding

Plant breeding is one of the most important components in securing food production and enhancing crop stability under both biotic and abiotic stress. It was developed since early 1900s and played the role in genetic selection and diversification for sustainable agriculture development (Fu, 2015). Modern plant breeding emphasized on maximizing crop yield under several circumstances but at the same time minimizing crop failure from environmental changes (Shahriari, et al., 2013). This process requires interpretation for genetic compatibility, relationship among inbred lines and utilization for diversified breeding resources (Mustafa, Saleh and Kashiani, 2021). Traditionally, crop genetic diversity was evaluated from their phenotypically traits, especially with the agro-morphological traits of interest to users. However, genetic diversity to our understanding was a term not defined clearly. It was widely understood as variation in nucleotides, genes or any other genetic material from the aspects of their taxonomical level. This general definition brought misinterpretation to the users and in fact, genetic variation among populations were reflected from their differences in allele and genotype frequencies (Fu, 2015). Those differences were outcome with variated performance from the targeted organisms regarding their changes from morphological measurements. Utilization on the genetic variations allowed humans to perform directional selection in a small population for enhancement of desired traits via the process known as modern plant breeding program. However, such process conducted in small population size

could lead to potential loss of genetic variation, which also known as genetic drifting and resulting in reduction of population fitness (Begna, 2021). As consequence, multiple breeding resources were suggested to improve population sizes that could reduce the effects from genetic drifting (Pekkala, et al., 2014).

Among the basis to conduct plant breeding program, the prerequisite was the selection of breeding resources for inbred line development. Breeding resources were the hybrid varieties or heterozygote groups with traits of interest performed that could be heritable upon genetic selection (Kashiani, et al., 2010). After that, inbred line development would be developed through successive generations of inbreeding followed by selection and testcross. Inbreeding triggered the fundamental genetic change that produced homozygosity and came with result of phenotypic changes (Ralls, Frankham and Ballou, 2013). Lastly, the inbred lines with desired traits would be crossbreed and achieving enhanced hybrid varieties through approaches of field test and market survey.

2.3.2 Sweet Corn Hybrid Breeding

Hybrid varieties are the first generation progeny produced by single-crossing unrelated parents through inbreeding. Inbreeding is a common technique conducted nowadays in both plant breeding and livestock breeding. It is a process used to breed unrelated homozygous parents together for the achievement of heterozygosity progeny with the desired traits remained (Begna, 2021). Homozygote parents could be achieved by self-pollination, but it might

cause genetic deterioration in the self-pollinated parents. Additionally, according to Berlan (2021), George (1908) conducted the first scientifically recorded corn inbreeding experiment. Initially, the corn inbreeding was conducted by self-pollination through several breeding cycles until their homozygous genetic purity higher than 99% (Berlan, 2021). After that, the inbred lines were utilized for single-crossing and resulted in single-crossed hybrid that outperformed their parents. The inbred lines are important and widely used because the single-crossed hybrid is found to have tremendous improvement regarding their yield count, fruiting quality or planting conditions (Pekkala, et al., 2014). This phenomenon is known as heterosis that hybrid progeny obtained having greater characteristics than mean value of their parents (Birchler, et al., 2010). On the other hand, sweet corn hybrid breeding could also be conducted in double-crossing of inbred line, but researchers had identified single-crossed hybrids performed better (Rani, et al., 2021). Hybrid breeding of sweet corn emphasized on alleles combinations from two homozygote unrelated parents which could be termed as inbred and their genetically identity were different from variations of alleles presented. In contrast, all progeny produced from single-crossing of the inbred would be genetically identical due to equal distribution of genetic material both unrelated inbred (Pekkala, et al., 2014).

2.3.3 Sweet Corn Inbred Lines

More than century, worldwide breeders has been developing commercial sweet corn hybrids through standard or conventional sweet corn breeding process

(Mena, et al., 2022). This process involved development of homozygous inbred lines through lab culture or continuous selfing for at least eight breeding cycles (Begheyn, Lubberstedt and Studer, 2016). As consequence, selection of desired phenotypes from hybrid sources for inbred line development is a crucial process in developing homozygosity with desired traits (Guan, et al., 2015). The method of selection could be conducted through pedigree selection from broad-based breeding populations which isolated the desired populations for selfing. After the inbred lines had been developed, they would be cross-breed and developing multiple hybrids to determine their commercial potential from farmers and consumers preferences. Sweet corn hybrids development would not only be going through site selection but also flavor screening (Lertrat and Pulam, 2007). The developed hybrids would be bite tasted at their marketing stage to filter the best hybrids produced by considering both fruit quality and their flavor and taste.

Sweet corn inbred line is the homozygote variety with desired traits preserved. It could be achieved through self-pollination or anther culture that both having distinct differences on time consumed. Self-pollination also known as selfing, it is a process of obtaining pollen from tassels and fertilizing ear from the same plant (Mabin, et al., 2021). Every selfing process in each breeding cycle reduced heterozygosity by 50% until 99% genetic homozygosity is achieved. However, selfing resulted in phenotypic deterioration of size and performance of sweet corn in every new successive generation therefore inbred was not ready for consumption (Amegbor, et al., 2022). Inbreeding often comes after selection of desired traits to preserve the genetic material.

There are four mutants that successfully utilized by sweet corn breeders from *sh2*, *bt*, *su* to *se*, and they were performed singly or in combination with each other for modern breeding program to create new commercial sweet corn varieties (Revilla, Anibas and Tracy, 2021). Lab-based breeding work has contributed to manipulation of these mutant genes that aided in controlling the sugar level in corn kernel. Recently, the sweet corn varieties were concluded into four main groups including standard sweet corn, supersweet, sugary enhanced and high sugar sweet corn (Revilla, Anibas and Tracy, 2021). Each variety contained unique combination of the mutated genes that resulted in desired traits, but there are still some drawbacks of these varieties. For instance, standard sweet corn and sugary enhanced varieties lost their sweetness rapidly after maturity therefore required emphasize on precision harvesting. As consequence, supersweet varieties are developed to resolve these issues. (Goncalves, et al., 2018). However, supersweet varieties were found to have reduced flavoring from their tough pericarp, lack creaminess and poor corn sweetness. The supersweet varieties were also relatively hard to germinate which caused high expenses for production. The youngest varieties developed were high sugar sweet corn that emphasized in solving all the problems (Goncalves, et al., 2018). However, better varieties that emphasized on superior kernel quality and agronomic performance were needed. Therefore, the urge for sweet corn breeding is increasing and it must be gaining more attention and support from researchers.

2.3.4 Heritability

Moreover, heritability is the crucial selection criteria while conducting traits selection for plant breeding. The desired traits must be associated with high heritability because it is a measurement for average effectiveness from genetic material contributed to phenotypic variance (Schmidt, et al., 2019). It helped to determine degree of resemblance between relatives therefore the inbred for desired traits with high heritability would be able to show the same traits again during cross-breeding. In shorter words, heritability is the extent to how much the phenotype is genetically determined. Heritability calculation was concluding findings from three aspects including expression of organisms' genotype, influences from environmental variances and interactions between both of them (Pazaran, 2019). The results would be expressing relationships between phenotypic values and phenotypic variance with genotypic values and genotypic variance.

Originally, heritability is proposed for animal breeding where the phenotypic and genotypic observations were collected from individuals (Wang, et al., 2013). Each individual from animal breeding usually have own and unique genotype that do not share with others. Therefore, genotype in heritability is referring to the single animal presented, and they could not be completely replicated in their environments. However, plant breeding was a process conducted with crop cultivars represented in large group of individual plants from exactly same genotype. Plants that derived from their vegetative propagation or reproduction process were considered clones, inbred lines, or hybrids having total same

genotype (Piepho and Mohring, 2007). As consequence, genotype in plant breeding referred to as the large group of individual plants sharing same cultivar which resulted in complete cloning of same genotypes (Lourenco, Ogutu and Piepho, 2020). Besides, the multiple observations from same cultivar are resulting in obtaining mean value for single phenotypic and genotypic variances.

In plant breeding, heritability can be measured in broad-sense (H^2) or narrow-sense (h^2) (Schmidt, et al., 2019). Broad-sense heritability is the measurement of proportion of phenotypic proportion as attribute to genetic causes emphasizing on how all genetic materials affect phenotypic differences (Mugisa, et al., 2022). However, narrow-sense heritability is influenced by additive gene and non-additive gene that is not conducted in this study. Increased selection efficiency could be achieved by heritable ratio of genetic variance to phenotypic variance with ranging from zero to one. Heritability values of 0.5 or higher could indicate the averagely half of the phenotypically differences from plants were influenced by genetic factor (Ranjan and Gautam, 2018). As consequence, the desired traits are suggested to be as high as possible to ensure traits inherited to succession generations.

2.3.5 Correlation among Traits

Plant breeding emphasized on desired traits selection that the selection process could be complex and difficult to conduct. However, the efficiency of traits selection could be broadened to selection of certain traits from genetic parameters estimation (Munawar, et al., 2013). This estimation is fundamental

to plant breeding as it helped to identify action of genes that involved in controlling quantitative traits. Therefore, selecting the traits having high correlations estimation to desired traits relating crop yield would result in ease of cultivar selection (Silva, et al., 2016). For example, sweet corn desirable traits that directly affecting the crop quality was their sizes and the sizes may only have low heritability. As consequence to preserve the desired sizing traits, other traits that having high correlation coefficient to the size of sweet corn could be chosen to preserve both the selected traits and corn sizes (Munawar, et al., 2013). Correlation could be understood as the co-relationship between two variables whereas correlation coefficient is the measurement for degree of association for the two variables. Correlation coefficient is presented in measurements from -1 to 1. Both positive and negative values were acceptable and the closer of coefficient value to -1 or to 1 were indication of stronger negative or positive correlation between the variables (Shyam, et al., 2018). As consequence, correlation coefficient is crucial to desired traits selection while conducting plant breeding to broaden the choices of traits.

2.3.6 Local Sweet Corn Breeding Program

Sweet corn is one of the cereal crop species that having most phenotypically variation (Kashiani and Saleh, 2020). Their phenotypic traits are easily influenced by abiotic factors such as weather, water conditions, light intensity and temperature. On condition of that, corn had developed variation of genetic diversity to have different water requirement in accordance, as consequence, their tolerance to environmental changes was highly succeed (Kashiani and

Saleh, 2020). Sweet corn could be short-listed as one of the value crops for replacement of vegetative crops in Malaysia that susceptible to climate changes and pest invasion (David, 2022). Their nature as a C4 plant emphasized on continuous utilization of sunlight as their energy sources for growth promoted their survivability in tropical environment (Mohammad, et al., 2022).

As the sweet corn's performance would be affected by abiotic factors that may affect traits selection during plant breeding process, molecular markers could be utilized to detect genetic variation among development of inbred lines for sweet corn (Fu, 2015). Developing inbred line and selection for hybrid varieties field trial were simple yet time consuming, labor intensive and costly process. In Malaysia, most seeds except for oil palm production were imported (Nazmi, et al., 2021). There were about 95% of sweet corn hybrid seeds being imported from the lack of plant breeding and seed producing industry in Malaysia (Ng and Tong, 2015). Despite the fact that plant breeding is a competitive business in Malaysia, this opportunity was not appreciated by the locals. Local plant breeding and seed production activities were mainly conducted and relied on government agencies such as Malaysia Agricultural Research and Development Institute (MARDI). The main factors behind this phenomenon included institutional weakness from lack of researchers and reliable weathering conditions for quality seed maturation (Ng and Tong, 2015). As consequence, sweet corn breeding process were not in much progress. Proposed solution to these issues were increasing plant breeding frequencies practiced by private sectors and diversity of crops breed.

2.4 Morphology of Sweet Corn Pollen

2.4.1 Study of Pollen Grains

Sweet corn is well known as wind pollinated plants as their pollen could be transported over distances through wind force (Anderson and Kulp, 1922). The study of sweet corn pollen played important role in archaeology and taxonomical identification for different sweet corn varieties (Halbritter, et al., 2018). This is because pollens from different species of plants had morphological variation (Danner, Hartel and Dewenter, 2014). Observation of sweet corn pollen could be conducted through light microscope or scanning electron microscope (SEM) that should encompass all structural aspects from the pollen grain (Halbritter, et al., 2018). The two approaches conducted to observe sweet corn pollen were to visualize shape, size, aperture number and location of the aperture for detailed comparison of their general features (Halbritter, et al., 2018). Whereas further studies on pollen stratification, development stages and wall layers could be conducted through transmission electron microscopy (TEM) or chromosome fluorescent staining approaches (Huang, et al., 2013).

2.4.2 Double Haploid for Sweet Corn Breeding

Traditional plant breeding method is conducted from continuous self-pollination of desired heterozygote varieties, but the approach is costly, labor intensive and time consuming (Kumar and Choudhary, 2020). In order to overcome these issues, modern plant breeding has been implementing anther

culture for the production of haploid cells and further stimulated into double haploid callus with colchicine that achieved 100% homozygosity in just one breeding cycle (Singh, Swapnil and Sihha, 2020). This process greatly reduces time consumed and increases selection efficiency. However, achieving double haploid culture induction is not a high success rate process itself. The crucial element for high success rate included donor plant, anther or pollen developmental stages, culture medium and culture environment (Pappammal, et al., 2019). Previous study emphasized that sweet corn could be achieving highest success rate of anther culture from utilizing developing pollen from early to late uninucleate stages as they were haploid microspore that could be stimulated for double haploid induction (Zhang, et al., 2013). As consequence, the stage of microspores identification should be conducted from observation of their external and internal structures (Ibrahim, et al., 2014). External structures observation could be conducted from SEM or light microscope analysis while chromosome fluorescent staining and TEM were suitable for internal analysis.

2.4.3 Sweet Corn Pollen Characterization

Sweet corn produces abundance of pollen grains that are heavier and larger than other anemophilous plant (wind pollinated) such as *Arabidopsis* (Wang, et al., 2017). This will greatly affect dispersal distance of sweet corn pollen; therefore, their planting distance plays vital role for successful pollination (Hofmann, Otto and Wosniok, 2014). Sweet corn is one of the essential model plants for monocotyledon pollen biology study as they are globally cultivated grain crops. The most accessible developmental stages of pollen are the maturity stages as

they could be visually observed. However, there are only few studies emphasized on synchronized sweet corn pollen development as the pollen development is enclosed inside the florets of the tassels and the developmental stages could be hardly predicted (Begcy and Dresselhaus, 2017). The size of sweet corn pollen grain is relatively large in anemophilous groups ranging from 80 to 125 μm diameter (Gong, Wu and Wang, 2015). They have singular pore and circular apertures as general characteristics for all corn species. Aperture is important on pollen as it aided in the formation of pollen tubes that favors releasing of male gametes for successful fertilization (Bozic and Siber, 2020).

2.4.4 Scanning Electron Microscope (SEM)

Observation of structural variations in pollen grains requires the aid from advanced equipment such as SEM. However, before the observation under SEM could be conducted, a series of conventional pretreatment process are required (Blackmore and Barnes, 1985). Firstly, the pollen grains need to be soaked in aldehyde overnight for cell fixation. Next, the pollen grains require to be dehydrated from series of ethanol concentration from low to high (Asahi, et al., 2015). The dehydrated pollen grains will be kept in freeze dryer for another night; therefore, they could be totally dried. Lastly, dried pollen grains will need to be metal sputtered from gold or platinum so the structural detail of the pollen grains could be reflected from the metal ions and presented in SEM observation panel (Tsuda, et al., 2014). However, these complicated and laborious procedures can possibly alter the morphology of pollen grains if they were not dehydrated correctly (Murtey and Ramasamy, 2016). Therefore, pollen grains

need to be dehydrated with series of ethanol from low to high concentration subsequently due to water particles would be causing structural changes when the pollen grains are observed in vacuumed SEM (Ceniceros, et al., 2014). In sum, the reviewed information will be utilized to analyze the current study and discuss the results.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental Design Outline

The overall layout of the current study is shown in Figure 3.1.

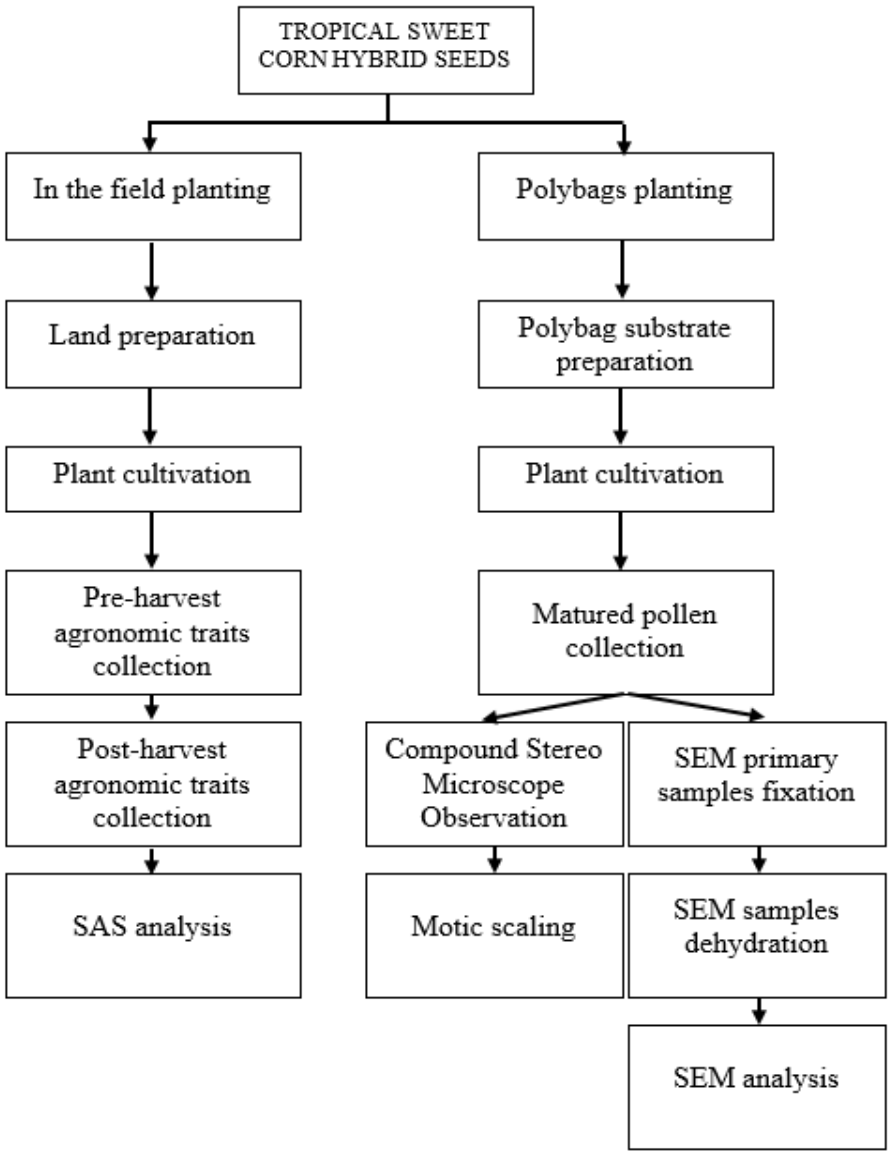

Figure 3.1: The overall layout of the current study.

3.2 Location of Study

The study was conducted in Universiti Tunku Abdul Rahman Kampar campus, Ag Park B. The decimal degrees coordinate for the location is 4.342921, 101.139804.

3.3 Materials and Equipment

3.3.1 Planting Materials

Five sweet corn imported hybrid seeds were obtained from the local agriculture shops in Kampar, Perak. Table 3.1 showed the name and population structure of the selected sweet corn hybrid varieties.

Table 3.1: List of tropical sweet corn hybrid used in the study.

Company	Variety	Source Population	Status of Breed
Agroniche	Big Bicorn	317	Hybrid Population
CropPower	Asia Manis	SS-932	Hybrid Population
GreenEagle	Jagung Manis Asia Best	1602	Hybrid Population
Green World Genetics (GWG)	Akik	Sc-422	Hybrid Population
Leckat	Sugar Rich	592	Hybrid Population

3.3.2 Chemical

Table 3.2: Chemicals and reagents list.

Chemicals and Reagents	Company	Country
1.8% Abamectin	Halex (M) Sdn. Bhd.	Malaysia
5.5% Cypermethrin	Wesco Agencies	Malaysia
Calcium nitrate ($Ca(No_3)^2$)	Yara International	Norway
Ethanol	SIME Scientific	Germany
Foliar 16:16:16	Yi Nong (M) Sdn. Bhd.	Malaysia
Foliar 6:6:6 (Boron)	Yi Nong (M) Sdn. Bhd.	Malaysia
Glutaraldehyde	Thermo Fisher Scientific	US
NPK 20:10:10 (Granular)	Yara International	Norway
NPK (Granular)	Yara International	Norway
Phosphate buffer saline (PBS)	ChemSol	US

3.3.3 Instrument

Table 3.3: Instruments list.

Instruments	Company	Country
Analytical Balance	Mettler-Toledo Inc	US
Brix Pocket Refractometer	ATAGO	Japan
Centrifuge Machine	Eppendorf	Germany
Compound Stereo Microscope	Moticam	China
Digital Vernier Caliper	Mitutoyo	Japan
Drying Oven	Binder GmbH	Germany
Measuring Tape (5 M)	MR.DIY	Malaysia
Micropipette	Eppendorf	Germany
Plastic Knapsack Sprayer (16 L)	Tonic Cap Gajah (M) Sdn. Bhd.	Malaysia
Scanning Electron Microscope (SEM)	JEOL	Japan
Tiller	Honda	Japan

3.3.4 Reagent Preparation

Abamectin pesticide solution

The abamectin pesticide solution was prepared by adding 5 mL of Halex Habamec 1.8 EC stock solution into 10 L of tap water to form a 2000X serial diluted pesticide solution.

Cypermethrin pesticide solution

The cypermethrin pesticide solution was prepared by adding 10 mL of Wesco Cyperin 550 stock solution into 10 L of tap water to form a 1000X serial diluted pesticide solution.

Ethanol solution

The 25% (v/v) ethanol solution was prepared by mixing 25 mL of absolute ethanol with 75 mL of distilled water.

The 50% (v/v) ethanol solution was prepared by mixing 50 mL of absolute ethanol with 50 mL of distilled water.

The 75% (v/v) ethanol solution was prepared by mixing 75 mL of absolute ethanol with 25 mL of distilled water.

The 95% (v/v) ethanol solution was prepared by mixing 95 mL of absolute ethanol with 5 mL of distilled water.

Glutaraldehyde solution

The 2.5% (v/v) glutaraldehyde stock solution was prepared by mixing a total of 0.5 mL of 25% (v/v) glutaraldehyde liquid stock with 4.5 mL of distilled water.

Phosphate buffer saline (PBS) solution

The phosphate buffer saline solution was prepared by solubilizing one phosphate buffer saline dehydrated tablet into 100 mL of sterile distilled water.

3.4 Agronomic Performance Analysis

3.4.1 Experimental Design

The experiment was conducted in Randomized Complete Block Design (RCBD) with three replications. The Figure 3.2 shows each replicate consists of five plots whereas each plot represented one tropical sweet corn hybrid variety.

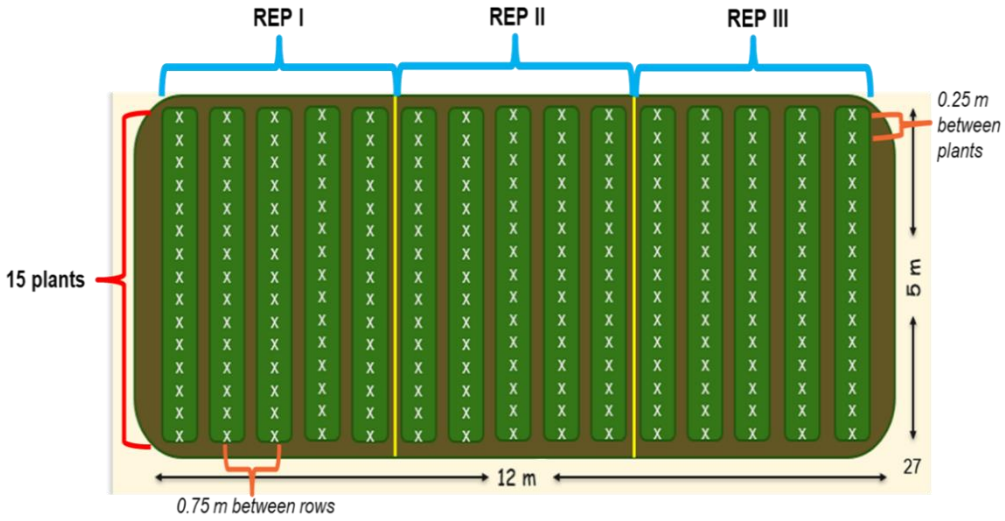

Figure 3.2: Planting layout of the study based on RCBD.

3.4.2 Land Preparation

A field with an area size of 60 m^2 was prepared by conducting weed removal and land tillage using a Honda tiller. The land was tilled repeatedly three times to practice a false seed bank approach. The EFB compost was applied to the soil during third tillage approach at the rate of 4000 kg/ha. After that, 15 soil beds were formed with trenches between each of them as shown in Figure 3.1. Each soil bed was prepared with a distance of 0.75 m and each plant was given 0.25 m of space in the soil bed.

3.4.3 Plant Cultivation

The seedling for field planting was prepared by germination in 108 slots germinating trays. The seeds were planted 2 to 3 cm in depth with three seeds per slot. After that, during day 10 of germination, the plants were thinned to one plant per slot and transferred into Ag Park B during the evening at about 6.00 pm as the soil was damped and temperature was cooled which would promote seedlings survival. A total of 12.0 x 5.0 m experiment area was prepared, and each individual plot was stand for variety. They were sowed in the tilled field and each plot was planted with 15 seedlings. The consistency was ensured by maintaining the planting distance at 0.75 m between rows and 0.25 m between plants within a row.

3.4.4 Fertilizer Application

The fertilizer applied were consisting of several dissimilar compound fertilizers from foliar to granular forms. The first fertilizer applied was urea using foliar spray, the urea granular was first dissolved in tap water with a rate of 10 g/L and applied to the leaves of sweet corn seedlings during their day 10 of growth from germination in seedling tray. After that, the seedlings were supplied with a 3:1 portion of NPK 15:15:15 and urea for each seedling during their day 15 of growth after germination. After that, the plants were supplied with foliar spray of NPK 16:16:16 and granular fertilizer of NPK 20:10:10 for every fortnight at day 29 and day 43 of growth after their germination. After day 45 of plant growth, the presence of tassels was observed and granular fertilizer of NPK 12:12:17 was supplied weekly. The silking process from the ear of the plant was observed during day 50 to day 55 of plant growth and the plant was foliar sprayed with NPK 6:6:6 that rich in calcium boron. Subsequently, the plants were continuously to be supplied with granular NPK 15:15:15 at day 55 and day 72 of plant growth to promote fruiting process.

3.4.5 Weed Control

The weeds were controlled manually by hand and mechanically using solarization approach. Hand weeding was conducted every week from day 20 of plant growth to remove or shorten the weeds surrounding the plants on the soil bed. The weeds height was maintained below the height of the sweet corn seedlings.

3.4.6 Irrigation

Plants were irrigated manually using garden hose with 30 m in length. They were watered once in a day during evening period. Irrigation was conducted in daily routine except for occurrence of raining weather. Leaves condition of the plant were frequently observed to ensure the plants stay out from water and heat stress with sufficient irrigation.

3.4.7 Pest Control

The main pest observed from the study were insect pests such as *Spodoptera frugiperda* and *Helicoverpa zea*. As consequence, pest control methods including chemical approach, biocontrol approach and mechanical approach were implied to the study. Firstly, chemical approach was conducted using pesticides, pesticides with abamectin and cypermethrin as active ingredients were applied rotationally to the plants every fortnight using a 16 L plastic knapsack sprayer in the evening starting from day 20 of plant growth. However, chemical approach was paused during day 48 of plant growth and biocontrol approach was conducted. The biocontrol agent from order of Odonata, Mantodea and Passeriformes were observed during the study and pest density was controlled with no harm spotted to the plants.

The plants in the field were for agronomic traits analysis purposes, therefore, mechanical approach such as physical barriers were build up to prevent attack from wild boar so the plants can grow well until they produce matured sweet corn. Dried palm leaves were placed around the physical barriers to prevent wild

boar from digging the barriers. Besides, synthetic tiger urine was hanged high around the physical barrier to trick the wild boar.

3.4.8 Harvesting and Sampling

Harvesting of the overall plants was conducted at about 20 days after silking, which falls under milky stages of plant growth. It was conducted manually using human hand. The ear was hold firmly, pulled down and followed by anticlockwise twisting to remove the ear from the plant. Three random individual plants were chosen from each plot for measurement of plant height, stem diameter, no. of leaves, ear height, tassel length, no. of husks/ears, dehusked ear weight, dehusked fresh ear yield, dehusked ear length, dehusked ear diameter, no. of kernels/rows, no. of kernel rows/ears, kernel length, total soluble solid, dehusked dried ear weight and dehusked dried ear yield. The data were collected before and after harvesting as followed their characteristics.

3.4.9 Agronomic Performance Data Collection

The agronomic performance of the tropical sweet corn hybrid varieties was evaluated from their traits. Pre-harvest and post-harvest traits were collected throughout the study period and the traits measured help to bring out the differences between varieties scientifically.

3.5 Pre-harvest Agronomic Traits Collection

3.5.1 Plant Height (cm)

Plant height was measured on the randomized sample plants with triplicates, as shown in Figure 3.3; it was measured from ground level to the node of the flag leaf base from the tassel.

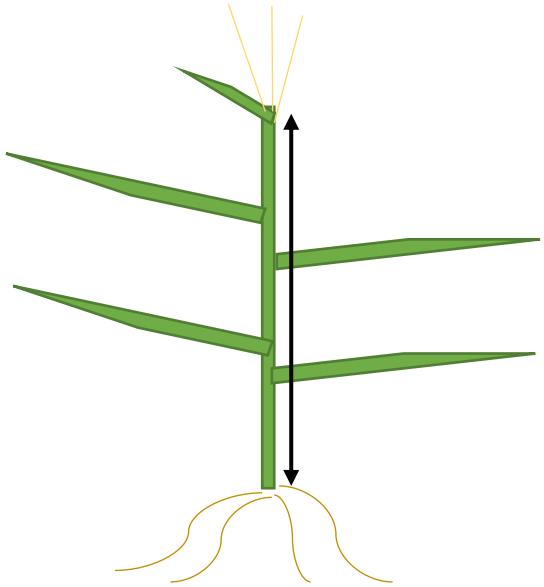

Figure 3.3: Plant height (cm) measurement for the study.

3.5.2 Stem Diameter (cm)

The stem diameter of the plants was measured in three points of the plant from bottom, middle and upper stem part of each individual plants and divided to represent stem diameter of each individual plant. Triplicates were measured for each variety in each plot using vernier caliper.

3.5.3 Leaves Number

The leaves number of the plant were concluded during their fruiting maturation stages counted from bottom to top leaves including flag leaves that surrounding the tassels. Triplicates were collected upon measurement of leaves number.

3.5.4 Ear Height (cm)

The ear height of each individual plant was measured from bottom of the plant touching the ground until husk node from the ear of the plant. The measurement was conducted in randomized and triplicate approach.

3.5.5 Tassel Length (cm)

The tassel length of the plant was measured from the node of the flag leaf base from the tassel until the top of the primary branches in the tassel. They were measured in triplicates and randomly obtained.

3.5.6 Number of Ears per Hectare

Number of ears per hectare were measured by conducting calculation on total ear that could be harvested from each plant multiply by number of plants that could be planted on one hectare of land and divided by one hectare.

3.6 Post-Harvest Agronomic Traits Collection

3.6.1 Number of Husks per Ear

The freshly harvested matured sweet corn were dehusked and each husk detached were counted. The total detached husks per ear were considered number of husks per ear. The dehusked ear were in triplicates during the measurement.

3.6.2 Dehusked Ear Weight (g)

After the ears had been dehusked, they were weighed on an analytical balance and their weight were recorded in g. Each variety in each block was measured in triplicate approach.

3.6.3 Dehusked Fresh Ear Yield (kg/ha)

The dehusked fresh ear yield was measured by multiplying average dehusked ear weight with number of plants that could be planted in one hectare and divided by hectare. The average dehusked ear weight was first converted into kg before conducting the calculation where the formula was:

$$Plants\ per\ acre = \frac{4046.86\ m^2}{0.1875\ m^2}$$

$$Yield\ (\frac{kg}{acre}) = \frac{Dehusked\ fresh\ ear\ weight\ X\ 21583\ (Plants\ per\ acre)}{1000}$$

3.6.4 Dehusked Ear length (cm) and Ear Diameter (mm)

Dehusked ear length was measured from bottom of the ear to top of ear using meter rule. In other hand, the ear diameter was measured in three points from the ear which consist of bottom, middle and top of the ear and took the average diameter as the ear diameter. The measurement was conducted from randomized triplicates of ear.

3.6.5 Number of Kernels per Row

The number of kernels per row was average number of kernels from randomized three rows in each sample ear.

3.6.6 Number of Kernel Rows per Ear

The number of kernel rows per ear was the total number of kernel rows on each sample ear and the average was taken from three randomized representative ear samples for each block.

3.6.7 Kernel Length (mm)

The kernel length was average length of kernel from three randomized representative in each sample ear. After that, the length was averaged again from three representative of ear sample from each block.

3.6.8 Total Soluble Solids Concentration (TSS) (%)

The total soluble solids concentration (TSS) was measured by squeezing three representative kernel's juices from each ear sample into 40 mL of distilled water. Each mixture was ensured to have 10 g of kernels mixing with 40 mL of distilled water and crushed with micropestle. After that, 1mL of the mixture was injected onto prism face of brix refractometer. The exact value was read and multiplied with five as the dilution factor. After each measurement, the prism face of brix refractometer was rinsed with distilled water and wiped with Kim wipes before next usage. This measurement was conducted within first 60 min from harvesting of the ears.

3.6.9 Dehusked Dried Ear Weight (g) and Ear Yield (kg/ha)

The dehusked ear samples were oven dried at 65°C in a drying oven for five days until they reached a constant weight. The dehusked dried ear samples were then transferred into a desiccator for cooling before weighing was conducted. When the dehusked dried ear samples reached room temperature (25°C), they were weighed on an analytical balance, and the dry weight of the samples was measured to calculate the dehusked dried ear yield of the sampled ears. The dehusked dried ear yield measurement was conducted by multiplied averaged dehusked dried ear weight with number of plants that could be planted in one hectare and divided by hectare. The average dehusked dried ear weight was first converted into kg before conducting the calculation.

3.7 Matured Pollen Morphology Collection

3.7.1 Experimental Design

The experiment was conducted in RCBD with three replications. Each replicate consists of five plots whereas each plot represented one tropical sweet corn hybrid variety.

3.7.2 Polybag Preparation

The 40 cm depth polybags were prepared in Ag Park B located in Kampar, Perak. A total of 50 polybags were prepared and each variety of sweet corn F_1 hybrid was distributed with 10 polybags. The polybags were placed in an open area beside Ag Park B greenhouse but with a physical barrier surrounding them to prevent an attack from wild boar.

Each polybag was filled three-quarters of the depth with a mixture of Baba Super10 organic planting soil and EFB compost. The ratio for the substrate mixture was three planting soil over one compost in volume. The substrate was well hydrated before planting.

3.7.3 Plant Cultivation

The seeds from five tropical sweet corn hybrid varieties were directly seeded into the prepared soil in polybags. Each variety was planted with 10 seedlings.

The plants were watered regularly with two watering sessions daily. The tassels' occurrence in the plants was spotted on day 42 of planting. The plants were planted for SEM purposes therefore the tassels were completely harvested without letting the silk be fertilized for sweet corn development.

3.7.4 Fertilizer Application

The fertilizer applied were consisting of granular form only. The only fertilizer applied was 2 g of granular NPK 15:15:15 for each seedling during their day 15 of growth after germination. After that, the plants were consistently supplied with granular NPK 15:15:15 in fortnight basis until harvesting the matured tassels for pollen collection.

3.7.5 Weed Control

The weeds were controlled manually by hand. Hand weeding was conducted every week from day 15 of plant growth to remove the weeds surrounding the plants in the polybags. The weeds were completely eradicated to prevent nutrient competition from enclosed planting system.

3.7.6 Irrigation

Plants were irrigated manually using garden hose with 30 m in length. They were watered twice in a day during early morning and evening period. Irrigation was conducted in daily routine without interference from weather conditions.

Leaves condition of the plant were frequently observed to ensure the plants stay out from water and heat stress.

3.7.7 Pest Control

The main pest observed from the study were insect pests such as *Spodoptera frugiperda*. As consequence, chemical approach was implied to the study. Firstly, chemical approach was conducted using pesticides, pesticides with abamectin and cypermethrin as active ingredients were applied rotationally to the plants every fortnight using a 16 L plastic knapsack sprayer in the late evening starting from day 15 of plant growth.

3.7.8 Pollen Sampling

Matured pollen from the plant were collected around day 40 to day 50 from plant germination by cutting matured tassels from plants in each variety. The tassels were placed onto a clean white paper and knocked 10 times. The florets that fell from the tassels were removed and yellow matured pollen was kept in 1.5 mL sterile microcentrifuge tubes. The collection of the matured pollen was conducted in triplicate for the subsequence study.

3.8 Matured Pollen Microscope Analysis

3.8.1 Compound Stereomicroscope Observation

The compound stereomicroscope observation was conducted according to protocol of Halbritter, et al. (2018). The mature pollen from each sweet corn F_1 hybrid variety was placed onto pre-cleaned microscope slides and stained with sterile distilled water. After that, square covering slips were placed onto the samples on slides. The matured pollen was observed under a compound stereo microscope with a Motic camera attached to the eyepiece of the microscope. A magnification level of 400X was used for observation and the images were received on a laptop. Scaling and contrast adjustments were performed to show clear images with the aid of Motic scaling computer application.

3.9 Matured Pollen SEM Analysis

3.9.1 SEM Sample Primary Fixation

The SEM sample preparation including sample primary fixation and dehydration were conducted based on procedures of Komai, et al. (2014) with slightly modification. The collected matured pollens were soaked overnight with 1 mL of 2.5% (v/v) glutaraldehyde solution for each variety in the 1.5 mL microcentrifuge tube. The soaked overnight samples were centrifuged at 1500 rpm for 1 min to obtain pellets by removing the supernatant. After centrifugation, all the pellets and supernatants were separated, and the pellets (sample) were collected. The pellets were then added with 1 mL of PBS solution for washing purpose and soaked for 10 min, followed by centrifugation at 1500 rpm for 1 min. The process of washing and centrifugation were repeated three times to obtain the cleaned samples. After that, the samples were washed with 1 mL of

sterile distilled water for 10 min, followed by centrifugation at 1500 rpm for 1 min. The washing and centrifugation processes were repeated three times as well.

3.9.2 SEM Sample Dehydration

A series of different concentrations of ethanol were prepared from 25%, 50%, 75%, 95%, and lastly 100%. The fixed samples were dehydrated by incubation in a series of ethanol. The samples were first dehydrated by adding 1 mL of 25% ethanol and incubated at room temperature (25°C) for 5 min, followed by centrifugation at 1500 rpm for 1 min to collect the pellets. The pellets were then dehydrated with 1 mL of 50% ethanol and incubated at room temperature (25°C) for 10 min. The samples were centrifuged at 1500 rpm for 1 min and the pellets were collected. The 1 mL of 75% ethanol was then added into each sample tube and incubated for another 10 min with only one attempt. The same centrifugation process was conducted, and 1 mL of 95% ethanol was added after the supernatant was removed and this process was repeated three times. Lastly, absolute ethanol incubation was conducted triplicate by adding 1 mL of absolute ethanol for the sample in each attempt. After the series of dehydration processes were completed, the absolute ethanol was removed, and the remaining absolute ethanol in the sample was evaporated until obtaining the powdery and whitish appearance for the pollens. The dehydrated specimens were then ready to be delivered to the SEM analysis laboratory for SEM observation.

3.10 Statistical Analysis

The agronomic performance analysis was conducted in triplicate to collect triplicate results which were reported as mean with standard deviation (SD) in the form of mean ± SD. Statistical analysis of the collected quantitative measurement for pre-harvest and post-harvest agronomic traits of the sweet corn F_1 hybrids were analyzed using Statistical Analysis System (SAS) On Demand to identify the correlation between the traits, the heritability of sweet corn varieties and mean and standard deviation for the quantitative data.

3.10.1 Effects of Blocks and Differences among Varieties

The analysis of variance (ANOVA) was conducted to evaluate performance of selected tropical sweet corn hybrid varieties under randomized complete block design. All the analysis in this study was conducted using General Linear Model coded as "PROC GLM" in Statistical Analysis System OnDemand. The key-out of ANOVA table were conducted to analyze the performance of varieties in the study and was shown in Table 3.4.

Table 3.4: ANOVA key out tests for the effects of blocks and sweet corn varieties.

Source of variation	Degree of freedom (df)	Mean squares (MS)	Expected mean square
Blocks	(r-1)	MS_v	$\sigma^2{}_e + i\,\sigma^2$
Varieties	(t-1)	MS_V	$\sigma^2{}_e + r\,\sigma^2$
Error	t (r-1)	MS_e	$\sigma^2{}_e$
Total	(rt-1)		

3.10.2 Broad-Sense Heritability

The heritability of the tropical sweet corn hybrid varieties was conducted to identify the total variation between individuals in a fixed population from the factor of genetic variation, it was suggested by Becker (1986) with following formula:

$$(h^2B) = \frac{\sigma^2 G}{\sigma^2 p}$$

$$\sigma^2 G = \frac{\sigma^2 + r\sigma^2 e - \sigma^2 e}{r}$$

$$= \frac{(MS_V - MS_e)}{r}$$

$$\sigma^2 p = \sigma^2 G + \sigma^2$$

$$= \frac{(MS_V\text{-}MS_e)+MS_e}{r}$$

h^2_B: Broad-sense heritability,

σ^2_G: Genotypic variance,

σ^2_P: Phenotypic variance,

σ^2_V: Variance for varieties,

σ^2_e: Environmental variance,

MS_V: Mean squares due to varieties,

MS_e: Error mean squares, and

r: Number of replications.

3.10.3 Pearson's Correlations

Simple correlation among the traits were determined from conducting calculation based on the formula from Gomez and Gomez (1984). This calculation was conducted using the software SAS OnDemand with formula:

$$r_{XY} = \frac{\sum[(X_i - X)(Y_i - \bar{Y})]}{\sqrt{\sum[(X_i - X)^2 x (Y_i - \bar{Y})^2]}}$$

Where:

r_{xy} = Correlation coefficient,

X_i = X value,

Y_i= Y value,

$\bar{X}$ = Mean value of character, X, and

$\bar{Y}$ = Mean value of character Y.

CHAPTER 4

RESULTS

4.1 Performance of Tropical Hybrid Sweet Corn Varieties

4.1.1 Analysis of Variance (ANOVA) for the Traits

Results of the analysis of variance were shown in Table 4.1 and it revealed that the effects of block and its interaction with hybrid varieties were significant only for the ear height (18.39), whereas the effects of hybrid varieties were found significant for plant height, no. of leaves on plant, ear height, tassel length, no. of husks/ears, no. of kernels/row, kernel length and lastly total soluble solids with mean square of 780.64, 4.50, 431.30, 61.28, 16.84, 37.53, 2.02 and 13.21 subsequently. The significant effects of varieties indicate that the hybrids evaluated varied in their performance.

Table 4.1: Mean squares in ANOVA for traits measured on five tropical hybrid sweet corn varieties.

		Mean square				
Source	DF	DEW	DDEW	PH	SD	NL
Block	2	528.53_{ns}	7.68_{ns}	8.05_{ns}	0.04_{ns}	0.12_{ns}
Varieties	4	1048.58_{ns}	32.49_{ns}	780.64^{**}	0.04_{ns}	4.50^{**}
Error	8	810.85	43.72	7.76	0.05	0.14
C.V.(%)		11.46	12.85	1.55	9.22	3.37

ns: not significant at $p > 0.05$, * significant at $p \leq 0.05$, ** significant at $p \leq 0.01$, DEW: Dehusked ear weight, DDEW: Dehusked dried ear weight, PH: Plant height, SD: Stem diameter, NL: No. of leaves.

Table 4.1: (continued).

		Mean square				
Source	DF	EH	TL	NHE	DEL	DED
Block	2	18.39^{**}	1.71_{ns}	0.03_{ns}	0.21_{ns}	0.01_{ns}
Varieties	4	431.30^{**}	61.28^{**}	16.84^{**}	1.71_{ns}	0.13_{ns}
Error	8	1.81	1.26	0.12	0.49	0.04
C.V.(%)		1.85	2.83	3.80	3.52	3.77

ns: not significant at $p > 0.05$, * significant at $p \leq 0.05$, ** significant at $p \leq 0.01$, EH: Ear height, TL: Tassel length, NHE: No. of husks/ears, DEL: Dehusked ear length, DED: Dehusked ear diameter.

Table 4.1: (continued).

		Mean square			
Source	DF	NKR	NKRE	KL	TSS
Block	2	2.94_{ns}	0.63_{ns}	0.01_{ns}	0.08_{ns}
Varieties	4	37.53^{**}	2.21_{ns}	2.02^{**}	13.21^{**}
Error	8	3.13	2.46	0.01	0.03
C.V.(%)		4.04	9.72	1.08	1.12

ns: not significant at $p > 0.05$, * significant at $p \leq 0.05$, ** significant at $p \leq 0.01$, NKR: No. of kernels/row, NKRE: No. of kernels rows/ears, KL: Kernel length, TSS: Total soluble solids.

4.1.2 Mean Comparison for the Characteristics Measured

The average performance of the sweet corn hybrid varieties were presented in (Table 4.2). From the separation of mean values indicated by DMRT, dehusked ear yields of the tropical hybrid sweet corn was revealed by Leckat sweet corn with the value of 5876.40 ± 462.52 kg/ha. However, it was not significantly higher than sweet corn from GreenEagle, GWG, Agroniche and CropPower (5656.04 ± 106.19, 5295.82 ± 482.38, 5116.90 ± 310.58 and 4876.46 ± 176.76 kg/ha, respectively). The highest dehusked ear weight among the sweet corn was found out to be Leckat sweet corn with a value of 272.27 ± 21.43 kg but had no significant difference from GreenEagle (262.06 ± 4.92 kg), GWG (245.37 ± 22.35 kg), Agroniche (237.08 ± 14.39 kg) and CropPower (225.94 ± 8.19 kg). Their dehusked dried ear weight were measured and Leckat sweet corn was discovered to have the highest dried weight recorded with value of 54.94 ± 3.51 kg but having no significant difference from sweet corn of GreenEagle

(50.56 ± 3.08 kg), GWG (51.29 ± 4.40 kg), Agroniche (53.95 ± 3.56 kg) and CropPower (46.54 ± 2.65 kg).

Table 4.2: Mean values ± standard deviation measured on five tropical sweet corn hybrid varieties evaluated.

Varieties	Traits				
	DEY (kg/ha)*	**DEW (kg)***	**DDEW (kg)***	**PH (cm)***	**SD (cm)***
Leckat	5876.40 ± 462.52^{A}	272.27 ± 21.43^{A}	54.94 ± 3.51^{A}	185.93 ± 2.46^{B}	2.49 ± 0.16^{A}
GreenEagle	5656.04 ± 106.19^{A}	262.06 ± 4.92^{A}	50.56 ± 3.08^{A}	193.08 ± 2.19^{A}	2.18 ± 0.13^{A}
GWG	5295.82 ± 482.38^{A}	245.37 ± 22.35^{A}	51.29 ± 4.40^{A}	173.28 ± 0.98^{C}	2.22 ± 0.09^{A}
Agroniche	5116.90 ± 310.58^{A}	237.08 ± 14.39^{A}	53.95 ± 3.56^{A}	192.95 ± 0.91^{A}	2.28 ± 0.10^{A}
CropPower	4876.46 ± 176.76^{A}	225.94 ± 8.19^{A}	46.54 ± 2.65^{A}	155.07 ± 0.65^{D}	2.32 ± 0.11^{A}

*Results are reported as the mean of triplicate measurements ± standard deviation (n = 3). Means followed by the same letter(s) in the same column are not significantly different at $p \leq 0.05$ based on DNMRT.

Table 4.2 (continued).

Varieties	Traits				
	NL*	**EH (cm)***	**TL (cm)***	**NHE***	**DEL (cm)***
Leckat	11.00 ± 0.00^{B}	63.77 ± 1.04^{D}	41.51 ± 0.60^{B}	12.67 ± 0.19^{A}	20.37 ± 0.57^{AB}
GreenEagle	12.78 ± 0.11^{A}	91.91 ± 1.41^{A}	44.66 ± 0.77^{A}	10.67 ± 0.19^{B}	19.38 ± 0.34^{B}
GWG	9.33 ± 0.38^{C}	63.07 ± 0.66^{D}	42.09 ± 0.76^{B}	7.89 ± 0.29^{C}	20.87 ± 0.49^{A}
Agroniche	10.78 ± 0.22^{B}	67.99 ± 0.92^{C}	33.75 ± 0.28^{D}	7.89 ± 0.11^{C}	19.46 ± 0.14^{B}
CropPower	10.89 ± 0.11^{B}	76.08 ± 2.05^{B}	36.12 ± 0.81^{C}	7.00 ± 0.00^{D}	19.08 ± 0.15^{B}

*Results are reported as the mean of triplicate measurements ± standard deviation (n = 3). Means followed by the same letter(s) in the same column are not significantly different at $p \leq 0.05$ based on DNMRT.

Table 4.2 (continued).

Varieties	Traits				
	DED (cm)*	**NKR***	**NKRE***	**KL (mm)***	**TSS (%)***
Leckat	5.17 ± 0.10^{A}	46.00 ± 0.88^{A}	16.11 ± 0.49^{A}	10.84 ± 0.00^{A}	15.10 ± 0.06^{C}
GreenEagle	5.15 ± 0.04^{A}	45.56 ± 0.73^{AB}	16.67 ± 0.38^{A}	9.55 ± 0.13^{D}	16.07 ± 0.11^{B}
GWG	4.65 ± 0.15^{B}	46.78 ± 0.95^{A}	14.67 ± 1.02^{A}	8.60 ± 0.02^{E}	12.94 ± 0.10^{D}
Agroniche	4.96 ± 0.11^{AB}	42.33 ± 1.20^{B}	16.78 ± 0.73^{A}	9.84 ± 0.00^{C}	16.95 ± 0.12^{A}
CropPower	5.03 ± 0.08^{A}	38.22 ± 1.22^{C}	16.44 ± 1.24^{A}	10.12 ± 0.01^{B}	11.97 ± 0.15^{E}

*Results are reported as the mean of triplicate measurements ± standard deviation (n = 3). Means followed by the same letter(s) in the same column are not significantly different at $p \leq 0.05$ based on DNMRT.

Moving on, the highest plant height presented in Table 4.2 were revealed by variety GreenEagle (193.08 ± 2.19 cm) but not significantly higher than Agroniche (192.95 ± 0.91 cm), however, it was significantly higher than Leckat (185.93 ± 2.46 cm) and GWG (173.28 ± 0.98 cm). Sweet corn from CropPower had been discovered to have significantly shortest plant height of 155.07 ± 0.65 cm. There was no significant difference acquired from stem diameter of the sweet corn varieties but Leckat was discovered to have highest stem diameter of 2.49 ± 0.16 cm among them. The next trait evaluated was number of leaves on the plant. GreenEagle was found to have highest number of leaves obtained with the value of 12.78 ± 0.11 leaves count. It was significantly higher than Leckat (11.00 ± 0.00 leaves count), CropPower (10.89 ± 0.11 leaves count) and Agroniche (10.78 ± 0.22 leaves count). Sweet corn from GWG was obtained with significantly lowest leaves count of only 9.33 ± 0.38 leaves identified.

Furthermore, the flowering part of plant were recorded with traits from ear height to tassel length of the plant. The ear height of the plant was found to be

significantly highest in GreenEagle (91.91 ± 1.41 cm) whereas the significantly shortest ear height was presented on Leckat and GWG with the value of 63.77 ± 1.04 cm and 63.07 ± 0.66 cm respectively. Plants from CropPower were found out to have significantly second highest ear height of 76.08 ± 2.05 cm and was significantly higher than Agroniche (67.99 ± 0.92 cm). Besides, tassel length of the plants from GreenEagle were discovered to have significantly longest tassel length of 44.66 ± 0.77 cm. The significantly moderate tassel length were shown by GWG and Leckat (42.09 ± 0.76 cm and 41.51 ± 0.60 cm) and they were performed better than CropPower (36.12 ± 0.81 cm) and Agroniche (33.75 ± 0.28 cm).

After the matured sweet corn were evaluated for their post-harvest traits, the very first evaluated post-harvest traits was the number of husk per ear and Leckat was revealed with significantly highest value of 12.67 ± 0.19 husks. The significantly lowest number of husk per ear was owned by CropPower with only 7.00 ± 0.00 husks counted. The significantly longest ear was identified to be GWG (20.87 ± 0.49 cm) and Leckat (20.37 ± 0.57 cm) whereas Agroniche, GreenEagle and CropPower (19.46 ± 0.14, 19.38 ± 0.34 and 19.08 ± 0.15 cm respectively) were significantly shorter than it. After the ear diameter were evaluated, the significantly highest dehusked ear diameter was revealed by Leckat (5.17 ± 0.10 cm) but having no significant difference from Green Eagle and CropPower (5.15 ± 0.04 and 5.03 ± 0.08 cm accordingly). GWG was discovered to have significantly lowest dehusked ear diameter of 4.65 ± 0.15 cm.

Moving on, the evaluation for number of kernels per rows was conducted, GWG was found to have significantly highest number of kernels per rows with the value of 46.78 ± 0.95 kernels/rows. However, it was not significantly higher than Leckat with a value of 46.00 ± 0.88 kernels/rows. CropPower in other hand was discovered to have significantly lowest count of number of kernels per rows with value of 38.22 ± 1.22 kernels/rows. In contrast, the number of kernel rows per ears were also evaluated and Agroniche was revealed with highest kernel rows/ears of 16.78 ± 0.73 kernel rows/ears but with no significant difference from GreenEagle, CropPower, Leckat and GWG (16.67 ± 0.38, 16.44 ± 1.24, 16.11 ± 0.49 and 14.67 ± 1.02 kernel rows/ears respectively). The kernels were removed from the sweet corn ears and evaluated for their kernel length. Among the five varieties of sweet corn, they were found to have all significantly differencing kernel length, but the significantly longest kernel length belonged to Leckat with value of 10.84 ± 0.00 mm recorded. The significantly shortest kernel length was revealed by GWG with value of 8.60 ± 0.02 mm. The last trait recorded was total suspended solid in the sweet corn. The total suspended solid from the varieties was found to be significantly different. The significantly highest total suspended solid count was revealed by Agroniche (16.95 ± 0.12%) while, the lowest total suspended solid count belonged to CropPower with value of 11.97 ± 0.15%.

4.1.3 Broad-Sense Heritability for Traits Measured on Different Varieties

The genotypic (σ^2_G) and phenotypic (σ^2_P) variances and broad-sense heritability (h^2_B) estimates for the pre- and post-harvest agronomic traits measured among the five tropical sweet corn hybrid varieties were evaluated and are as shown in

Table 4.3. Among all the traits, the total suspended solid was discovered to be the most heritable traits in the hybrid varieties with heritability estimates of 99.32%, it was followed by ear height (98.75%), kernel length (98.53%), number of husk per ear (97.89%), plant height (97.08%), tassel length (94.08%) and number of leaves (91.21%). Subsequently, number of kernel per row was found to be above moderate with heritability estimates value of 78.56%. The moderate broad-sense heritability estimates were acquired by dehusked ear length (45.35%) and dehusked ear diameter (42.86%), whereas lowest estimates were obtained from dehusked ear weight (8.90%). In other hand, the traits with negative heritability estimates were revealed by number of kernel row per ear (-3.51%) and stem diameter (-7.14%).

Table 4.3: Genotypic variances (σ^2_G), phenotypic variances (σ^2_P) and broad-sense heritability estimates (h^2_B) for all traits measured on five tropical sweet corn hybrid varieties.

	σ^2_G	σ^2_P	h^2_B (%)
Dehusked ear yield	79.24	890.09	8.90
Plant height	257.63	265.39	97.08
Stem diameter	0.00	0.05	-7.14
Number of leaves	1.45	1.59	91.21
Ear height	143.16	144.97	98.75
Tassel length	20.01	21.27	94.08
Number of husks per ears	5.57	5.69	97.89
Dehusked ear length	0.41	0.90	45.35
Dehusked ear diameter	0.03	0.07	42.86
Number of kernel per row	11.47	14.60	78.56
Number of kernel row per ear	-0.08	2.38	-3.51
Kernel length	0.67	0.68	98.53
Total suspended solid	4.39	4.42	99.32

4.1.4 Phenotypic Correlations

Results for phenotypic correlations among the traits measured on tropical hybrid sweet corn varieties were presented in Table 4.4. Plant height was found to be moderately correlated with no. of husks/ears, no. of kernels/row and highly correlated with total soluble solids, with the correlation coefficients of 0.544,

0.561 and 0.943 respectively. Next, the no. of leaves on the plant was discovered to be moderately correlated with dehusked ear diameter (0.556); highly correlated with ear height (0.821); negatively correlated with dehusked ear length (-0.549). The tassel length of the plant in other hand was found to be moderately correlated with no. of husks/ears and highly correlated with no. of kernels/row with correlation coefficient of 0.580 and 0.642 respectively.

When the traits come into post-harvest agronomic traits, the first trait with simple correlation coefficients analyzed was no. of husks/ears, it was discovered to not only having correlation with plant height and tassel length but also moderately correlated with no. of kernels/row, kernel length and dehusked ear weight (0.547, 0.519 and 0.544, respectively). Furthermore, the dehusked ear length was moderately correlated with no. of kernels/row, dehusked ear weight and dehusked dried ear weight with correlation coefficient of 0.565, 0.594 and 0.547 accordingly. Subsequently, the dehusked ear diameter was having moderately correlation with dried ear weight (0.570) whereas highly correlation with no. of kernels rows/ears and kernel length were discovered with correlation coefficient of 0.766 and 0.653 respectively. The no. of kernels/row was found to be negatively correlated with no. of kernels rows/ears (-0.509). Lastly, the dehusked ear weight and dehusked dried ear weight was found to be highly correlated to each other with correlation coefficient of 0.811. On the contrary, the only traits that had no correlation with any other traits measured was revealed by stem diameter with no significant correlation coefficient presented.

Table 4.4: Simple correlation coefficients among traits measured on tropical sweet corn hybrid.

Traits	PH	SD	NL	EH	TL	NHE	DEL	DED	NKR	NKRE	KL	TSS	DEW
SD	-0.043												
NL	0.405	-0.096											
EH	0.146	-0.213	0.821**										
TL	0.234	-0.094	0.289	0.306									
NHE	0.544*	0.258	0.460	0.081	0.580*								
DEL	0.057	0.334	-0.549*	-0.489	0.350	0.184							
DED	0.198	0.250	0.556*	0.399	0.098	0.489	-0.189						
NKR	0.561*	-0.087	-0.042	-0.126	0.642**	0.547*	0.565*	-0.286					
NKRE	0.130	0.094	0.328	0.259	-0.101	0.047	-0.216	0.766**	-0.509*				
KL	0.058	0.476	0.372	-0.032	-0.224	0.519*	-0.264	0.653**	-0.287	0.341			
TSS	0.943**	-0.001	0.483	0.191	-0.017	0.437	-0.141	0.292	0.306	0.279	0.196		
DEW	0.335	0.346	0.092	0.080	0.435	0.544*	0.594*	0.570*	0.312	0.332	0.167	0.234	
DDEW	0.386	0.486	-0.152	-0.189	0.051	0.270	0.547*	0.358	0.157	0.271	0.137	0.331	0.811**

**,*Significant at p = 0.01, p = 0.05, respectively, ns: not significant, PH: Plant height, SD: Stem diameter, NL: No. of leaves, EH: Ear height, TL: Tassel length, NHE: No. of husks/ears, DEL: Dehusked ear length, DED: Dehusked ear diameter, NKR: No. of kernels/row, NKRE: No. of kernels rows/ears, KL: Kernel length, TSS: Total soluble solids, DEW: Dehusked ear weight, DDEW: Dehusked dried ear weight.

4.2 Matured Pollen Morphology Analysis

4.2.1 Compound Stereomicroscope Observation of Matured Pollen

The light microscopic observation from Figure 4.1 indicated the sweet corn pollen grains were round-shaped and their sizes were relatively similar regarding genetic variances. The sizes of the pollen were observed with approximately 100 µm for every hybrid. There was minimal structure degradation of the pollen, and no fungi contamination were observed.

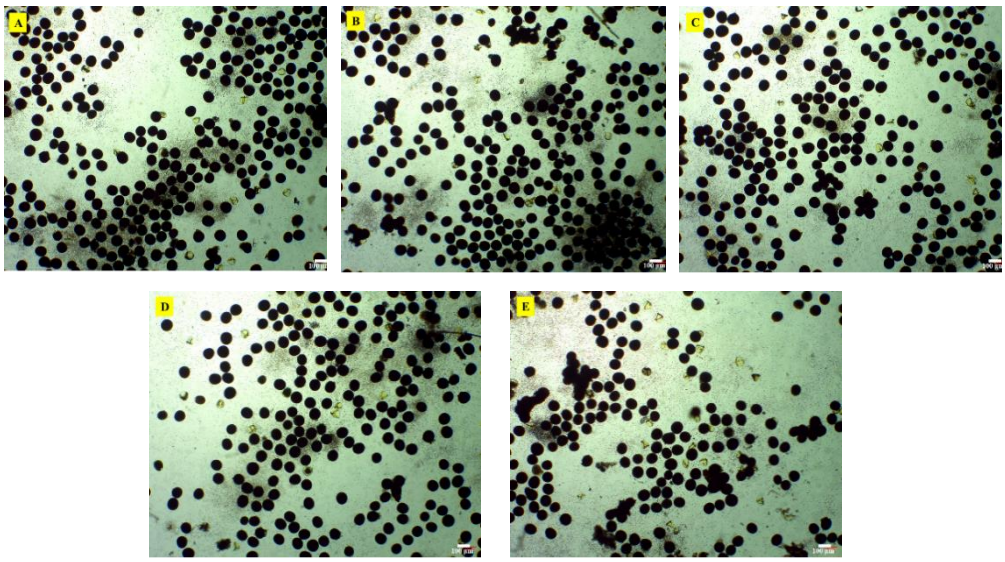

Figure 4.1: Pollen light micrograph; (A) Agroniche, (B) CropPower, (C) GreenEagle, (D) GWG, (E) Leckat; Scale bar = 100 µm.

4.2.2 SEM Analysis of Matured Pollen

The SEM analysis were shown in Figure 4.2. The pollen grains in the figure were characterized as monoporate that having singular pore, circular and non-prominent apertures. Respectively, enlarged single pollen have shown the surface detail of pollen grain showing operculum covering the single aperture in different variety. Overall, the ornamentation of exine and germination pore of sweet corn pollen grain from five different varieties were considered very similar to each other regarding their germination pore and exine morphology.

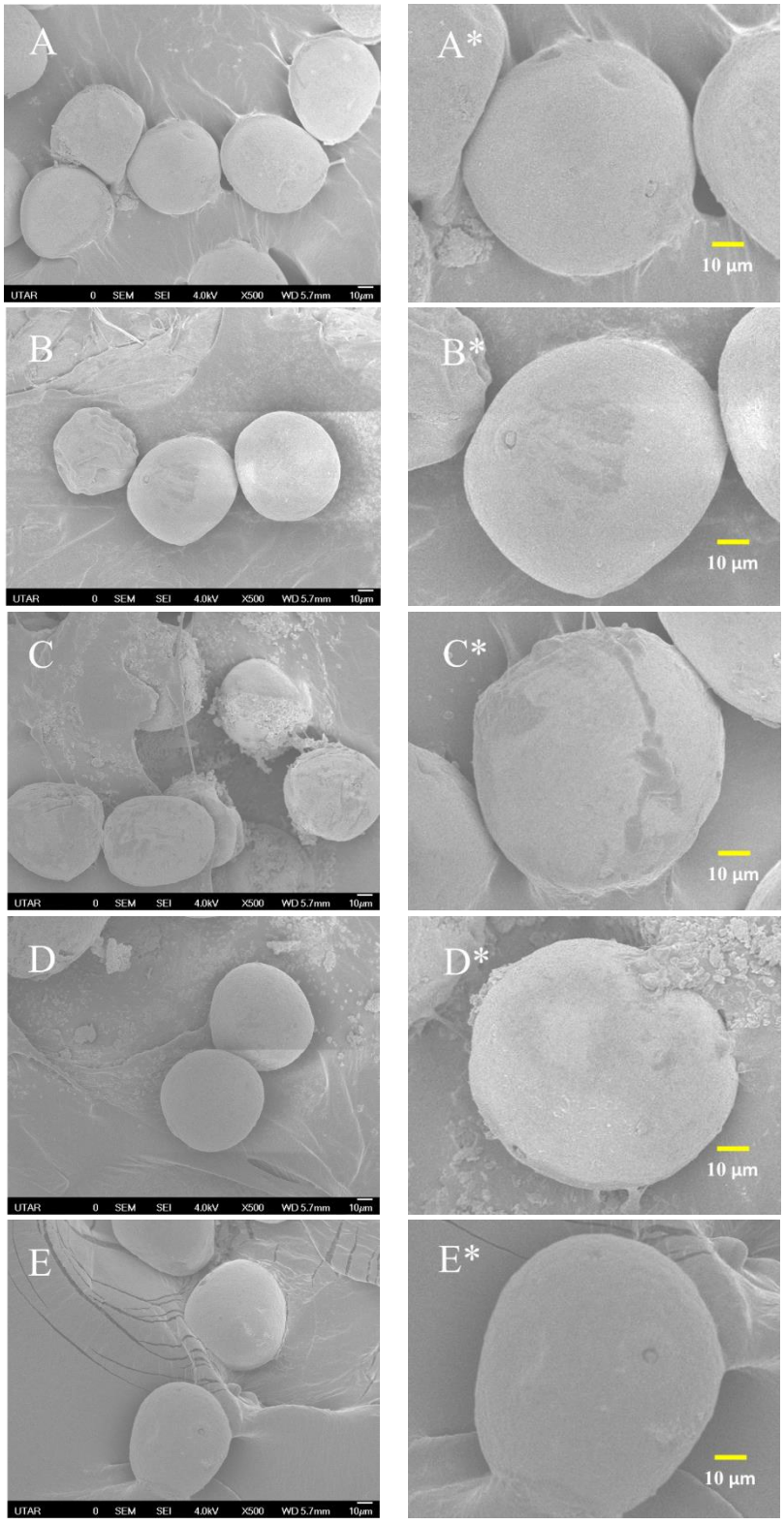

Figure 4.2: Pollen scanning electron micrograph (SEM); (A) Agroniche, (B) CropPower, (C) GreenEagle, (D) GWG, (E) Leckat; * for respective enlarged single pollen; Scale bar = 10 µm.

CHAPTER 5

DISCUSSION

5.1 Performance of Tropical Hybrid Sweet Corn Varieties

5.1.1 Genetic Variability

The result from analysis of variance in Table 4.1 revealed the existence of significant variability among tropical sweet corn F_1 hybrid for several traits in all blockings. The combined analysis of tropical hybrids for 14 traits of the F_1 sweet corn in Table 4.1 showed that the mean square of hybrid varieties and environments was significant for several studied traits. Nevertheless, the interaction between hybrids and environments in this study was not significant for all the traits except for the ear height which performed significant variations. A related study from Muliadi, Effendi and Azrai (2021) reported similar results that the effects of block were non-significant except for only one trait, the ear height was identified to have significant variation between hybrid and the environment. The insignificant for most of the traits evaluated from effects of block in this study as shown in Table 4.1 indicated no significant variation was obtained in the direction of blocking. Even though randomized complete block design (RCBD) was conducted to reduce errors from undesirable and uncontrollable factors such as soil fertility or environmental condition due to small sample sizes (Sewenet, 2019). The small sample sizes resulted in the small

area of study interfered with the present experiment (Stansluos and Kodaz, 2020). These observations indicated in most cases, environmental conditions had not appreciably influenced the expression of characters association (Begum, et al., 2012).

Conversely, the significant differences in measured traits between hybrid varieties were evaluated and shown in Table 4.1. The existence of these differences among the hybrids for eight of the traits indicated the presence of prominent genetic variability among the hybrids under study. Similar studies from Kashiani, et al. (2014), Munawar, et al. (2013) and Nordin, et al. (2018) also stated there is a significant variation between the measured traits and the varieties of sweet corn. Consequently, varied phenotypic performances from different varieties could be exploited for selection purposes in plant breeding programs (Subaedah, Edy and Mariana, 2021). In addition, promising genetic diversity enabled the improvement of varied quantitative and qualitative traits through the selection of elite F_1 hybrids exhibiting desirable phenotypic yielding traits that fulfilled breeding goals (Kandel, et al., 2018). According to FAMA (2022), sweet corn is graded by its length, diameter, and weight; hence, the ideal yielding traits would be traits that positively and significantly correlated with them.

Eventually, the mean of several traits measured from the imported hybrids could be compared for the identification of good yield traits and were shown in Table 4.2. The Table 4.2 shows that hybrid GreenEagle 1602 Super Sweet had the

highest plant height and ear height, namely 193.08 cm and 91.91 cm, respectively. According to studies from Wang, et al. (2023) and Zhao, et al. (2022), they stated that optimal range of good ear-plant height ratio is 36.60% to 39.43%, whereas, Muliadi, Effendi and Azrai, (2021) stated good ratio falls at 50%, which indicated the location of the ear would be in the middle of plant height. The hybrid variety having the highest percentage in this study was CropPower 932 Asia Manis (49.06%), which is similar to the study from Muliadi, Effendi and Azrai, (2021).

Another study from Saiin, et al. (2023) reported that the plant height of GWG 422 Akik obtained is 170.34 cm which is similar to the result of 173.28 cm obtained in this study. Based on the good ear-plant height ratio calculated, the ratio obtained for this variety was fall in the range of 36.60% to 39.43% as reported by Wang, et al., (2023). As for the remaining hybrids from Leckat 592 Sugar Rich (34.30%), GreenEagle 1602 Super Sweet (47.60%) to Agroniche 317 Big Bicorn (35.24%) were out of the optimal range of 36.60% to 39.43% as reported by Wang, et al., (2023) and is lower than the ratio (50%) reported by Muliadi, Effendi and Azrai, (2021). In sum, the good ear-plant height ratio of sweet corn obtained in this study is in the range of 34.30% to 49.06%. Both Wang, et al. (2023) and Zhao, et al. (2022) stated that the optimal range of ear-plant height ratio should be taken into consideration when breeding or selecting high-yielding hybrids due to optimal ear height is crucial for the plant to maximize utilization of fertilizer, moisture, and photosynthetically active radiation. Therefore, an optimal range of good ear-plant height ratio should be

considered for the enhancement of corn yields during the selection of high-yielding hybrids.

Moreover, results of the combined analysis (ANOVA and DMNRT) from Table 4.1 and Table 4.2 respectively indicated Leckat 592 Sugar Rich hybrid was showing the highest ear yield, namely 5876.40 kg/ha, followed by GreenEagle 1602 Super Sweet hybrid (5656.04 kg/ha). Good yield characteristics including the number of husks per ear, dehusked ear length, dehusked ear diameter, number of kernels per row, and number of kernel rows per ear promoted the high yield of these hybrids. Zakariah (2018) stated that inbred lines derived from single-cross hybrid source populations outperformed other kinds of populations regarding yield. As a consequence, hybrid varieties in this study with good yielding performance have good potential for developing superior inbred lines through continuous selfing or double haploid technique. As an imported source population, the superior inbred lines developed from them would be able to single cross with the local inbred line to bring out a new production of local hybrid varieties that could resolve issues of current local hybrids lacking sweetness and undesirable growth traits (Nordin, et al., 2018).

5.1.2 Broad-Sense Heritability Estimates

Heritability estimates are an essential tool for the breeding of quantitative traits, it provides statistically supporting evidence on the extent to which a particular trait that can be inherited by successive generations. In the present study, low estimates (less than 30%), moderate estimates (30 to 60%), and high estimates

(more than 60%) of heritability (h^2_B) were revealed by several traits measured and shown in Table 4.3, as defined by Johnson, et al. (1955). The Table 4.3 has shown that all the traits evaluated were found to have expressed high to moderate broad-sense heritability except for dehusked ear yield, dehusked dried ear yield, stem diameter, and number of kernel rows per ear. Among them, dehusked dried ear yield (-9.36%), stem diameter (-7.14%), and number of kernel row per ear (-3.51%) were found to have negative heritability estimates presented, which was rare cases in plant breeding program (Steinsaltz, Dahl and Wachter, 2020).

Previous reports by Belay (2018) and Ribeiro, et al. (2016) showed matching readings for plant height, ear height, and number of kernels per row from high heritable estimates found whereas Freeman, et al. (2019) for dehusked ear length and diameter in moderate estimates. A high heritability estimate indicated a high proportion of variation obtained from a character was affected by genetics, and low environmental effects were influencing the expression of these characters (Muliadi, Effendi and Azrai, 2021). As a consequence, the increase in performance in these traits was based on their phenotypic performance. High estimates of heritability for the variables suggest the variations were passed down to progeny. Hence selection for those traits with high heritability estimates from the hybrid varieties would be most effective for the expression of the traits in the development of inbred line genotypes (Kashiani, et al., 2014). Those high broad-sense heritability estimates for several quality traits in this study indicated selection of hybrid varieties with increased plant height, number of leaves, ear height, tassel length, number of

husks per ear, and total suspended solids, is possible to improve yield production. However, other factors influence the selection of hybrid varieties such as the influence of additive and non-additive genes. High heritability estimates alone could not validate the high genetic progress without considering other factors. Therefore, the indication of simple selection on high heritability does not necessarily represent the high genetic obtained in the progeny (Najeeb, et al., 2009). Therefore, Magar, et al. (2021) suggested that the heritability estimate in conjunction with genetic advance could be applied to greatly increase the effectiveness of selection for superior genotypes after the inbred line developed.

Moreover, three negative estimates as shown in Table 4.3 were obtained in the present study and it could be a sensible description of the data by indicating individuals from the same hybrid varieties (similar genotype) tend to have more phenotypic variation in their traits as compared to individuals from other hybrid varieties (random pairs) (Steinsaltz, Dahl and Wachter, 2020). Steinsaltz, Dahl and Wachter (2020) also stated that negative estimates could be happened due to faulty experimental protocols or materials that the phenomenon would cease to exist once the necessary correction was implemented. There is a study that reported that crop production conducted with seeds containing low genetic purity would have resulted in the segregation of the crop phenotypic traits within the similar genotype (Pallavi, et al., 2011). Thus, the cause of these negative estimates could have possibly resulted from low seed purity from either one or several hybrid varieties presented in this study. Therefore, the tropical sweet corn F_1 hybrid seeds were suggested to have conducted

International Seed Testing Association (ISTA) quality certification to achieve 99% of genetic purity (Milivojevic, Ripka and Petrovic, 2018).

In contrast, Qureshi and Khalil (2019) reported similar results of low heritability estimates for the yield. Low heritability was an indication of influence from environmental factors in the material tested. The corn yield is a multigene trait controlled by multiple loci dispersed over the corn chromosomes (Kashiani, et al., 2014). Gusmini, Wehner and Donaghy (2007) and Akbar, et al. (2008) provided the same evidence that segregation of heterozygous loci from the hybrid varieties during meiosis stages led to low heritability for corn yield. During the segregation process, the hybrid varieties would have segregation of alleles located in multiple loci but each loci has a small effect, however, environmental factors influenced multiple alleles thus, leading to low heritability. For this reason, corn yield phenotypic characteristics could be easily influenced by environmental factors, therefore, direct selection of characters that lead to low heritability estimates was not suggested. Among the environmental factors in this study included the amount of fertilizer input, heat stress, water stress, and pest attacks. The cultivated area played a minor role in affecting the corn yield as the blocking effects in this study were considered significantly low. In advance, better management methods such as fertilizer application and irrigation would need to be implied consistently to enhance the trait performance during hybrid planting; whereas for inbred line development, characters that are significant and positively correlated to the corn yield would be suggested as an indirect selection approach (Tilahun, et al., 2014).

5.1.3 Phenotypic Correlation Coefficient

Likely, selection strategies could be established regarding the correlation analysis among measured agronomic traits (Table 4.4). The Table 4.4 has shown that dehusked ear weight showed positive and significant phenotypic correlation with fresh and dried plant yields components such as dehusked dried ear weight (r = 0.811), dehusked ear length (r = 0.594), dehusked ear diameter (r = 0.570) and number of husks per ear (r = 0.544), subsequently. Among them, dehusked dried ear weight was the most weight-attributing trait, followed by dehusked ear length, dehusked ear diameter, and number of husks per ear. A positive and significant phenotypic correlation coefficient indicated the changes between the two variables were in the same direction (Kharel, et al., 2017). It could be further elaborated from examples such as the higher value of a particular variable was associated with the increased value from the other specific variables and vice versa. As a consequence, simultaneous selection conducted within these traits could enhance sweet corn production from increased weight.

Similar studies from Bartaula, et al. (2019) and Magar, et al. (2021) had shown matching results for dehusked ear length and dehusked ear diameter regarding their correlation coefficient evaluated due to higher measurements from dehusked ear length and diameter indicating larger ear is, which therefore proposed greater weight of the ear. Moreover, Amegbor, et al. (2022), Izzam, et al. (2017) and Singh and Kumar (2017) confirmed that ear aspects such as ear length, diameter, and husk count contributed to corn yield most directly;

therefore, their positive and significant correlations indicated these traits could be utilized for selecting high-yielding genotypes. Dehusked dried ear weight was having significantly highest positive correlation coefficient to dehusked ear weight as the dried ear weight was derived from oven drying of the fresh ear weight. Consequently, a high and positive correlation coefficient between them was as expected. On the other hand, the significant and positive correlation coefficient between the number of husks per ear with the dehusked ear weight could be caused by the effect of the number of husks on weight loss of the ear weight. Turkay and Karaman (2022) stated that the highest weight loss of ear weight would have resulted from the ear with the least husk count. Husk helped to reduce the water loss of sweet corn therefore maintaining sweet corn weight from high temperature and respiration rate (Liu, et al., 2021).

Besides, husks also played a crucial role in resisting ear damage by pests such as corn earworm (Lepidoptera: Noctuidae). Corn earworms caused high yield loss due to feeding habits that damaged the kernels and ears with the consequence of reduction in ear weight (Rector, Snook and Widstrom, 2003). Similarly, Amegbor, et al. (2022) reported husks count per ear should be considered as essential secondary traits of selection to prevent exposure of kernels from adverse environment and pest attack that would affect sweet corn yield and quality. Significant correlation values between those traits would be suggesting information that the traits were controlled by multiple tightly linked genes or manipulated by genes with pleiotropic effect (Naharudin, Sin and Saleh, 2021). The pleiotropic effect emphasized on increasing or decreasing performance from the particular traits would directly affect the trait of interest

(Pixley and Bjarnason, 1993). Hence, dehusked ear length, dehusked ear diameter, and number of husks per ear as shown in Table 4.4 would be suggested for an indirect selection of genotypes for dehusked ear weight due to the positive and high correlation between them.

5.2 Matured Pollen Morphology Analysis

5.2.1 Compound Stereomicroscope Observation of Matured Pollen

In this study, the matured pollen grains were obtained in early the morning from their anthers during the seventh to eighth weeks after planting and they were processed with observation immediately to preserve their structure. This is because the release of pollen grains for sweet corn could be started from sunrise until noon depending on environmental conditions such as temperature, humidity, or genetic constitutions (Kaefer, et al., 2016). Additionally, pollen collection is highly dependent on the environment's temperature and humidity, which would affect the pollen viability and structure (Bujang, Zakaria and Ramaiya, 2021). Therefore, the collection of pollen in the early morning was conducted to minimize the effects of temperature and humidity (Bujang, Zakaria and Ramaiya, 2021). In the present study, the pollen grains of sweet corn from different hybrids were hydrated in a drop of distilled water on a glass slide and then only observed under a compound microscope and the result was shown in Figure 4.1. The pollen stored in individual well-sealed microcentrifuge tubes was observed with a circular shape with few numbers of transparent pollens, besides there was no fungi contamination observed under

the light microscope. The Figure 4.1 shows the results obtained were similar to the research of Jayaprakash (2017), in which the pollen observed was circular. This result indicated there is least structural degradation occurred in the pollen and they were free from fungi contamination (Halbritter, et al., 2018). If there is structure degradation or fungi contamination, the pollen observed will be an abnormal appearance of an exine surface or with the presence of fungal spores surrounding as reported by Kostic, et al. (2019). Moreover, a preliminary step of observation of hydrated pollen under the light microscope was conducted before SEM analysis, this is to ensure the material was not degraded or contaminated by fungi that would affect the subsequent SEM results (Halbritter, et al., 2018).

Furthermore, the sizes of the pollen grains observed are shown in Figure 4.1 and were approximately 100 μm with minimal variation from their shapes, which is circular. The results were reported similarly from studies of Gong, Wu and Wang (2015) and Hofmann, Otto and Wosniok (2014) that who stated that sweet corn is wind pollinated plant with pollen grains that ranged from 80 to 125 μm diameter which the results obtained (approximately 100 μm) were lie within the interval. Based on the studies of Wang, et al. (2017), it shown that the size range in pollen grains of wind-pollinated plants (17-58 μm) was normally smaller than animal-pollinated plants (5-200 μm) as the pollen grains would need to be dispersed from wind force; therefore, they must be light and small structurally. However, the sweet corn pollen grains have resulted in larger diameters as compared to other wind-pollinated plant pollen grains. Hence, the larger sizes of sweet corn pollen might affect their dispersal range and therefore, they were

required to be planted in high density to ease the dispersion of large pollen grains (Hofmann, Otto and Wosniok, 2014). Another study by Ferreira, et al. (2007) also reported that sweet corn pollen grain does not have much strength when dispersed; therefore, rapid loss of viability within one to four hrs could be happened. As a consequence, sweet corn pollen grains were larger in size to enhance the effects of gravity forces to pull the pollen grains downwards to the silks on the ear that are located lower from the tassels (Gong, Wu and Wang, 2015). In addition, An, et al. (2019) mentioned that healthy sweet corn pollens should be swelled, regular round-shaped with no folded surface which is similar to the pollen observed in this study. In light of this, the selection of sweet corn varieties with healthy pollen grains was an essential process in plant breeding as sweet corn pollen is crucial for promising yield production (Choe, Ko and Williams, 2021). As a result, the pollen grains from all hybrid varieties as shown in Figure 4.1 in this study were observed preliminary and they could be short-listed as having healthy pollen since they have regular sizes and shapes with minimal variation.

5.2.2 SEM Analysis of Matured Pollen

Eventually, the pollen grains from different hybrids were observed under SEM to determine structural morphology and variations (Figure 4.2). Before the SEM was conducted, pollen samples would require a series of preparation from cell fixation to dehydration. Cell fixation ensured the sample remained in the same structural condition because the pollen would start to change morphologically after they were removed from anthers due to loss of water content (Williams,

2021). The dehydration process was conducted immediately after cell fixation to remove water molecules from the sample as the biological structure of pollen would shrink, collapse and break due to induced surface tension from water loss while the samples were observed in a vacuumed chamber inside the SEM (Fischer, et al., 2013). Lastly, the samples were coated with a thin layer of gold or platinum to ensure the conductivity of the samples that prevented build-up charges in the samples that influenced SEM results (Williams, 2021).

Studies from Wang, et al. (2022) and Zhang, et al. (2020) reported that sweet corn pollen normally shows only one aperture capped with an operculum, which was a similar observation for pollen grains of all hybrids in the present study as shown in Figure 4.2. Dresselhaus, Lausser and Marton (2011) stated that sweet corn as a model plant for the study of pollen development in monocotyledon produced pollen grains with a single distal aperture; whereas, Tsou, Cheng and Walden (2015) emphasized that eudicots produced pollen with three to six equatorial apertures. The monosulcate pollen grains produced by sweet corn could be characterized by a single furrow-like aperture that is commonly present on pollen surfaces (Albert, et al., 2018).

Among all the other pollen features, apertures played important roles in accommodating pollen deformation from changing hydration factors, controlling water and gas exchange for pollen grains, and participating in pollen performance from survival to germination of pollen tubes (Albert, et al., 2018; Vieira and Feijo, 2016). The presence of apertures was enabling the formation

of pollen tubes for fertilization which is crucially linked to yield performance from the crop of interest (Choe, Ko and Williams, 2021). As a consequence, the pollen grains for all hybrids in this study could be considered qualifying for fertilization due to the presence of aperture and operculum which are crucial in successful pollination. Operculum as presented in the apertures of all hybrid varieties for the study was for the function of blocking exogenous gene entry that could be causing transfection (Wang, et al., 2022). Besides, the operculum also protects the delicate apertural area to ensure the normal development of pollen tubes after germination was triggered (Furness and Rudall, 2006; Yang, et al., 2017). Consequently, the presence of both apertures and operculum was important for successful kernel formation each kernel on the corn cob represented each pollen fertilization (Zhou, Juranic and Dresselhaus, 2017).

In contrast, the abnormal development of apertures and operculum might lead to adverse effects such as pollen tube could not establish or genetic infection from external sources which negatively affect the growth of sweet corn and eventually resulting low quality and low yield production. Briefly, pollen grains from all hybrids in the present study were observed with minimal variations regarding their structure or sizes. Hence, hybrid varieties with the presence of smooth pollen surface, and apertures followed by evident operculum would be suggested for selection that could greatly induce successful pollination and resulted in promising yield quality.

5.3 Future Recommendation

This study was separated into two sections which are agronomic characteristics analysis and pollen morphology observation for different tropical sweet corn hybrids. Agronomic characteristics analysis was focused on evaluating the traits collected regarding their differences among varieties, heritability of traits, and correlation of performance between each trait whereas pollen morphology observation was conducted to determine pollen structure of sweet corn and their function to yield production. The RCBD was conducted to reduce the environmental error that could occur that influence the agronomic traits study in the area of study. However, results from analysis of variance (ANOVA) that indicated no significant effects of block reflected the unnecessary of conducting RCBD.

Besides, the error degree of freedom in RCBD was also smaller than the complete randomized design (CRD) which would result in a large error term if too much variation occurred between experimental units within a block (Alkutubi, 2021). Consequently, a future study conducted on the same location and same spot would be recommended to be conducted in CRD that does not account for the effects of soil fertility or uncontrollable environmental conditions (Mohr, Wilson and Freund, 2022). The small study area in the present study contributed to the low effects of soil fertility and environmental conditions as the variation of uncontrollable factors in smaller areas was considered lower.

Moreover, the sample sizes of the present study were considered small regarding the study on population genomics (Nazareno, Bemmels, Christopher and Lohmann, 2017). A future study is recommended to be conducted with more plant varieties with higher duplicates within each variety presented to greatly improve the sample size. As the sample size increases, the variability of the sampling distribution would be decreased; therefore, large samples would transform minimal differences into statistically significant differences that would strengthen the result (Orthod, 2014). Increasing sample size also allowed further analysis of genetic dissimilarity and selection index for the agronomic characteristics that would enhance the genotype selection process exploited for plant breeding purposes (Maleki, et al., 2019).

Furthermore, another recommendation would be suggested is the pollen development stages could be further studied using DAPI staining approach (Huang, et al., 2013). This is because the traditional inbred method required seven generations of selfing to achieve 99% of homozygosity; whereas double haploid inbred takes only one generation to achieve 100% homozygosity (Begheyn, Lubberstedt and Studer, 2016). As a consequence, the double haploid inbred method for a shortened duration of inbred line development would be increasing the efficiency of plant breeding. However, the double haploid technique for sweet corn inbred line development requires microspores from specific stages such as mid to late uninucleate stages (Zhang, et al., 2013). To determine the development stages of pollen, DAPI staining approaches could be considered.

CHAPTER 6

CONCLUSION

To sum up, the agronomic traits of different tropical sweet corn hybrid varieties were evaluated. The insignificant differences in performance from block effects indicate that there was minimal environmental variation such as soil fertility or environmental conditions in the area of the present study conducted. Whereas the significant differences in the majority of performance indicate that the hybrids varied substantially among themselves for multiple traits measured. These significant variations among the hybrids showed adequate genetic variations, hence, these differences could be exploited for specific purposes such as inbred line development in breeding programs. Based on the agronomic traits, the hybrids could be divided into two main groups those with high yield and high ear quality and those with low yield and low ear quality. This indicates the varied heterotic groups among the hybrids and those with high yield have great genetic values as a source population for potential inbred line development. The broad-sense heritability indicates yield in this study is a multigene trait based on low heritability obtained. Therefore, phenotypic correlation was conducted to identify traits significantly and positively related to yield with moderate to high heritability for indirect selection. Among the traits, the number of husks per ear, dehusked ear length and dehusked ear diameter were suggested for traits indirect selection purposes aiming to improve yield in successive generations. Therefore, Leckat 592 Sugar Rich hybrid could be shortlisted as

the source population for inbred line development aiming to improve yield in Kampar, Perak, and similar environments.

In pollen observation, the matured pollen from different hybrids was expected to have minimal variations as they were not different species of interest. The matured pollen observed under a light microscope and SEM in this study were having similar sizes, structures, and shapes, therefore, their pollen morphology was not interfered with by their genetic variations. However, source populations producing pollen with a healthy structural appearance of single aperture, granular operculum covering the aperture, and circular swelled shapes were suggested as pollen is crucial for promising yield production. Overall, matured pollen from all the hybrids in this study was observed with a healthy structural appearance and they would be suggested as a qualified source population for the breeding program.

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APPENDICES

Appendix A

Result of Analysis of Variance (ANOVA) for the traits measured.

Plant Height (cm)

Source	DF	Mean Square	Pr > F
Block	2	8.05	0.3973_{ns}
Varieties	4	780.64	$<.0001^{**}$
Error	8	7.76	
C.V.%	1.55		

ns: not significant at $p \leq 0.05$; ** significant $p \leq 0.01$.

Stalk Diameter (cm)

Source	DF	Mean Square	Pr > F
Block	2	0.04	0.4225_{ns}
Varieties	4	0.04	0.4847_{ns}
Error	8	0.04	
C.V.%	9.22		

ns: not significant at $p \leq 0.05$; ** significant $p \leq 0.01$.

Number of Leaves

Source	DF	Mean Square	Pr > F
Block	2	0.12	0.4547_{ns}
Varieties	4	4.50	$<.0001^{**}$
Error	8	0.14	
C.V.%	3.37		

ns: not significant at $p \leq 0.05$; ** significant $p \leq 0.01$.

Ear Height (cm)

Source	DF	Mean Square	Pr > F
Block	2	18.39	0.0063^{**}
Varieties	4	431.30	$<.0001^{**}$
Error	8	1.81	
C.V.%	1.85		

ns: not significant at $p \leq 0.05$; ** significant $p \leq 0.01$.

Tassel Length (cm)

Source	DF	Mean Square	Pr > F
Block	2	1.71	0.3108_{ns}
Varieties	4	61.28	$<.0001^{**}$
Error	8	1.26	
C.V.%	2.83		

ns: not significant at $p \leq 0.05$; ** significant $p \leq 0.01$.

Number of Husks per Ear

Source	DF	Mean Square	Pr > F
Block	2	0.03	0.7862_{ns}
Varieties	4	16.84	$<.0001^{**}$
Error	8	0.12	
C.V.%	3.80		

ns: not significant at $p \leq 0.05$; ** significant $p \leq 0.01$.

Dehusked Ear Length (cm)

Source	DF	Mean Square	Pr > F
Block	2	0.21	0.6663_{ns}
Varieties	4	1.71	0.0619_{ns}
Error	8	0.49	
C.V.%	3.52		

ns: not significant at $p \leq 0.05$; ** significant $p \leq 0.01$.

Dehusked Ear Diameter (cm)

Source	DF	Mean Square	Pr > F
Block	2	0.01	0.7252_{ns}
Varieties	4	0.13	0.0549_{ns}
Error	8	0.04	
C.V.%	3.77		

ns: not significant at $p \leq 0.05$; ** significant $p \leq 0.01$.

Number of Kernels per Row

Source	DF	Mean Square	Pr > F
Block	2	2.94	0.4295_{ns}
Varieties	4	37.53	0.0018^{**}
Error	8	3.13	
C.V.%	4.04		

ns: not significant at $p \leq 0.05$; ** significant $p \leq 0.01$.

Number of Kernel Rows per Ear

Source	DF	Mean Square	Pr > F
Block	2	0.63	0.7813_{ns}
Varieties	4	2.21	0.5076_{ns}
Error	8	2.46	
C.V.%	9.72		

ns: not significant at $p \leq 0.05$; ** significant $p \leq 0.01$.

Kernel Length (mm)

Source	DF	Mean Square	Pr > F
Block	2	0.01	0.6472_{ns}
Varieties	4	2.02	$<.0001^{**}$
Error	8	0.01	
C.V.%	1.08		

ns: not significant at $p \leq 0.05$; ** significant $p \leq 0.01$.

Total Soluble Solid (%)

Source	DF	Mean Square	Pr > F
Block	2	0.08	0.1009_{ns}
Varieties	4	13.21	$<.0001^{**}$
Error	8	0.03	
C.V.%	1.12		

ns: not significant at $p \leq 0.05$; ** significant $p \leq 0.01$.

Dehusked Ear Weight (g)

Source	DF	Mean Square	Pr > F
Block	2	528.53	0.5467_{ns}
Varieties	4	1048.58	0.3497_{ns}
Error	8	810.85	
C.V.%	11.46		

ns: not significant at $p \leq 0.05$; ** significant $p \leq 0.01$.

Dehusked Dried Ear Weight (g)

Source	DF	Mean Square	Pr > F
Block	2	7.68	0.8420_{ns}
Varieties	4	32.49	0.5888_{ns}
Error	8	43.72	
C.V.%	12.85		

ns: not significant at $p \leq 0.05$; ** significant $p \leq 0.01$.

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FACULTY OF SCIENCE

Full Name(s) of Candidate(s)	Yong Yan Shee
ID Number(s)	20ADB04545
Programme / Course	Bachelor of Science (Hons) Agricultural Science
Title of Final Year Project	Agronomic Characteristics and Pollen Morphology Among Tropical Sweet Corn Hybrid Varieties

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Signature of Co-Supervisor

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