



**UNIVERSITI PUTRA MALAYSIA**

***DIVERSITY, PERFORMANCE AND SELECTION OF TROPICAL SWEET  
CORN INBRED LINES, AND THEIR COMBINING ABILITIES IN HYBRID  
COMBINATIONS***

**NEYAZ RASHID MUSTAFA**

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By

**NEYAZ RASHID MUSTAFA**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

**November 2019**

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## **DEDICATION**

*I would like to dedicate with love to*

***My Beloved Father and Mother***

***Rashid Mustafa***

***Saadia Ahmad***

***My siblings***

***My Dearest Wife***

***Zubaidah Faiq***

***And***

***My Childern***

***Yara and Yad***



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**DIVERSITY, PERFORMANCE AND SELECTION OF TROPICAL SWEET CORN INBRED LINES, AND THEIR COMBINING ABILITIES IN HYBRID COMBINATIONS**

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**NEYAZ RASHID MUSTAFA**

**November 2019**

**Chairman : Professor Ghizan Saleh, PhD**  
**Faculty : Agriculture**

Local composite varieties of sweet corn offer moderate yields and eating quality, while imported hybrid varieties are less adaptable to the local environment, although having high eating quality. Therefore, the objectives of this study were (1) to evaluate the potential of 27 tropical sweet corn inbred lines developed from different source populations; (2) to determine genetic diversity among those inbred lines based on agronomic performance and simple sequence repeat (SSR) DNA markers; (3) to identify major heterotic groups among the inbred lines for selection of potential parents for hybrid production; and (4) to evaluate combining abilities among selected parental inbred lines in a half-diallel cross. Performance and heterosis of the single-cross hybrids were evaluated at two different locations, Field 2 and Field 10, Universiti Putra Malaysia, Serdang, Selangor. The genetic distances among the inbred lines as revealed by the SSR DNA markers, were used to predict hybrid performance.

A total of 27 near-homozygous sweet corn inbred lines were used in this study, comprising of 25 inbred lines which were developed through continuous selfing and selection from eight source populations, two previously developed inbred lines, and a commercial hybrid variety (Hybrid 530) used as a control, were evaluated for performance. Significant variations in agronomic performance were recorded among the sweet corn inbred lines. Among the inbred lines, the highest husked fresh ear yields were obtained from FTT-1, NTS-2, HSE-2, TNT-1, HAW-4, FTT-3, TNT-2, HAV-3, HAW-5 and HAV-2, with mean values of 15,875, 15,406, 15,237, 14,722, 14,105, 13,607, 13,556, 13,543, 13,284 and 13,248 kg ha<sup>-1</sup>, respectively. High genetic diversity was found among the inbred lines evaluated, indicating their good potential for utilization towards production of single cross hybrids.

Genetic diversity among the sweet corn inbred lines was also investigated using 100 SSR DNA markers. Among the SSR DNA markers amplified, 77 were found to be polymorphic. Results showed that the SSR markers were informative in detecting genetic differences among the sweet corn inbred lines, as exhibited by high Shannon's information index (I), Nei's expected heterozygosity (Nei's), and polymorphic information content (PIC), with mean values of 0.915, 0.546 and 0.474 respectively. Among the SSR markers amplified, bnlgl447, bnlgl152, umcl867, bnlgl839, umcl858, umcl805 (Appendix 1), umcl030, bnlgl036 and bnlgl176 were found to be the most informative to exhibit genetic diversity in the inbred lines, with PIC values of 0.732, 0.696, 0.682, 0.662, 0.653, 0.638, 0.629, 0.618 and 0.612, respectively. Shannon's information index, Nei's gene diversity coefficient and PIC showed that Chromosome 8 was the most informative chromosome ( $I = 1.060$ , Nei's = 0.619 and PIC = 0.544). The inbred lines were further separated into five diverse heterotic groups, based on phenotypic evaluations and six distinct groups based on molecular data. However, since phenotypic and molecular data of the inbred lines were independent of each other, six inbred lines, FTT-1, HAW-1, NTS-2, HSE-4, HAV-2 and EE0-2, were selected as parents based on the molecular data, each from different heterotic groups, to be utilized in a half-diallel cross scheme for the production of single-cross hybrids.

Among the hybrids evaluated, hybrids H11, H12 and H15 were found to have the highest husked fresh ear yields in Field 2 (22364, 21252 and 21906 kg ha<sup>-1</sup>, respectively), Field 10 (21356, 20393, 22623 kg ha<sup>-1</sup>, respectively), and the locations combined (21860, 20823 and 22265 kg ha<sup>-1</sup>, respectively). Hybrids that produced high yields were those formed between high × high, high × low or low × low yielding inbred crosses and from genetically diversified population sources. In addition, hybrids H11, H12 and H15 had high and consistent mid- and better-parent heterosis estimates for yield and most yield related traits, indicating that they have accumulated favorable genes acquired by their parental inbred lines.

Results of combining ability analysis showed significant additive and non-additive gene actions involved in the control of the traits measured. However, additive gene effects were found to be more important in the control of yield and yield components among the hybrids. Among the parental inbred lines, FTT-1, EE0-2 and HAW-1 had the highest positive and significant GCA effects for husked fresh ear yield in Field 2 (1,415.89, 1,132.22 and 777.89, respectively), Field 10 (975.22, 1,321.89 and 1,204.89, respectively), and the two locations combined (1,195.56, 1,227.06 and 991.39, respectively). This is an indication that these parental lines were the best general combiners among the parental inbred lines for high yield and yield components in the crosses involving them. Among the hybrids produced, P1 × P4 (H3), P4 × P6 (H13), P2 × P4 (H7) and P5 × P6 (H15) were identified as the best combinations, with favorable positive SCA estimates for most of the traits measured. The ratios of GCA to SCA variance (GCA/SCA) were found to be more than 1 for most of the traits measured. This has revealed that both additive and non-additive gene actions were important in the control of these traits although additive gene action was more pronounced.

The high broad-sense heritability estimates for most of the traits measured at both and combined locations, is due to a relatively small influence of the environment to the phenotypes. High narrow-sense heritability estimates obtained for most of the traits measured at both locations, indicate the predominance of additive gene actions in the inheritance of all these traits.

Results also showed that there was no association between genetic similarities among the six parental sweet corn inbred lines, as revealed by all the SSR DNA markers amplified, and hybrid performances, indicating that the genetic similarities based on SSR markers revealed in the present study were not indicative in predicting hybrid performances.

In conclusion, the three best performing hybrids, H11, H12 and H15 were each produced from a combination of two of the three superior inbred lines, FTT-1, EE0-2 and HAW-1. These inbred lines have proven to consistently show positive and significant GCA effects for yield and most yield related traits. Thus, these potential hybrids should be further tested in large scale multi-locational trials, before they could be released as new tropical sweet corn hybrid cultivars.

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

**KEPELBAGAIAN, PRESTASI DAN PEMILIHAN WARISAN TROPIKA  
INBRED DAN KEUPAYAAN BERGABUNG DALAM PENGELUARAN  
HIBRID JAGUNG MANIS**

Oleh

**NEYAZ RASHID MUSTAFA**

**November 2019**

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Varieti-varieti komposit jagung manis tempatan memberikan hasil dan kualiti pemakanan yang sederhana, manakala varieti-varieti hibrid yang diimport kurang kesesuaiannya pada persekitaran tempatan, walaupun mempunyai kualiti pemakanan yang tinggi. Oleh itu, objektif kajian ini adalah untuk (1) menilai potensi 27 titisan inbred jagung manis tropika yang dibangunkan daripada sumber populasi yang berbeza; (2) menentukan kepelbagaian genetik di kalangan titisan inbred tersebut berdasarkan prestasi agronomi dan penanda DNA SSR; (3) mengenalpasti kumpulan heterotik utama di kalangan titisan inbred tersebut dalam pemilihan induk yang berpotensi bagi pengeluaran hibrid; dan (4) menilai keupayaan bergabung di kalangan titisan inbred induk terpilih dalam kacukan separuh-diallel. Prestasi dan heterosis dari hibrid kacukan tunggal telah dinilai di dua lokasi yang berbeza, Ladang 2 dan Ladang 10, Universiti Putra Malaysia, Serdang, Selangor. Jarak genetik antara titisan inbred seperti yang ditunjukkan oleh penanda DNA SSR, telah digunakan untuk meramalkan prestasi hibrid.

Sejumlah 27 titisan inbred jagung hampir-homozigot telah digunakan dalam kajian ini, terdiri daripada 25 titisan inbred yang dibangunkan melalui pendebungaan sendiri dan pemilihan yang berterusan keatas lapan populasi sumber, dua titisan inbred yang dibangunkan sebelumnya, dan satu varieti hibrid komersil yang digunakan sebagai kawalan (Hybrid 530), telah dinilai prestasinya. Variasi yang ketara dalam prestasi agronomi telah direkodkan di kalangan titisan inbred jagung manis tersebut. Di kalangan titisan inbred yang dikaji, hasil tongkol tanpa kulit tertinggi telah diperolehi dari FTT-1, NTS-2, HSE-2, TNT-1, HAW-4, FTT-3, TNT-2, HAV-3, HAW-5 dan HAV-2, masing-masing dengan nilai min 15,875, 15,406, 15,237, 14,722, 14,105, 13,607, 13,556, 13,543, 13,284 dan 13,248 kg ha<sup>-1</sup>. Kepelbagaian genetik yang tinggi telah didapati di kalangan titisan inbred yang dinilai, menunjukkan potensinya yang baik untuk digunakan ke arah pengeluaran hibrid kacukan tunggal.



Kepelbagaian genetik di kalangan titisan inbred jagung manis juga telah dikaji menggunakan 100 penanda DNA SSR. Di kalangan 100 penanda DNA SSR yang telah diamplifikasi, 77 didapati polimorfik. Keputusan menunjukkan bahawa penanda SSR tersebut adalah sangat bermaklumat dalam mengesan perbezaan genetik di kalangan titisan inbred jagung manis tersebut, sepertimana dipamerkan oleh nilai indeks maklumat Shannon (I) yang tinggi, nilai keheterozigotan yang dijangka Nei (Nei's) yang tinggi, dan kandungan maklumat polimorfik (PIC) yang tinggi, masing-masing dengan nilai purata 0.915, 0.546 dan 0.474. Dalam kalangan penanda SSR yang diamplifikasi, bnlgl1447, bnlgl1152, umc1867, bnlgl1839, umc1858, umc1805, umc1030, bnlgl1036 dan bnlgl1176 didapati paling bermaklumat, bagi menerangkan kepelbagaian genetik dalam titisan inbred tersebut, dengan nilai PIC masing-masing 0.732, 0.696, 0.682, 0.662, 0.653, 0.638, 0.629, 0.618 dan 0.612. Indeks maklumat Shannon, pekali kepelbagaian gen Nei dan PIC menunjukkan bahawa Kromosom 8 adalah kromosom yang paling bermaklumat ( $I = 1.060$ ,  $Nei's = 0.619$  dan  $PIC = 0.544$ ). Titisan inbred tersebut seterusnya telah dipisahkan kepada lima kumpulan heterotik yang pelbagai, berdasarkan kepada penilaian fenotip, dan enam kumpulan yang ketara berdasarkan kepada data molekul. Walaubagaimana pun, oleh kerana data fenotip dan molekul titisan inbred adalah bebas antara satu dengan lain, enam titisan inbred, FTT-1, HAW-1, NTS-2, HSE-4, HAV-2 dan EE0-2 telah dipilih sebagai induk berdasarkan kepada data molekul, masing-masing dari kumpulan heterotik yang berbeza, untuk digunakan dalam skema kacukan separuh-diallel bagi pengeluaran hibrid kacukan tunggal.

Di kalangan hibrid yang dinilai, hibrid H11, H12 dan H15 telah didapati mempunyai hasil tongkol segar yang tertinggi di Ladang 2 (masing-masing dengan 22364, 21252 dan 21906 kg ha<sup>-1</sup>), di Ladang 10 (masing-masing 21356, 20393 dan 22623 kg ha<sup>-1</sup>), dan pada gabungan lokasi (masing-masing 21860, 20823 dan 22265 kg ha<sup>-1</sup>). Hibrid yang memberi hasil yang tinggi adalah yang terbentuk dari kacukan antara induk titisan inbred yang bersifat hasil tinggi x tinggi, tinggi x rendah, atau rendah x rendah, dan dari sumber populasi yang berbeza. Selain daripada itu, hibrid H11, H12 dan H15 mencatatkan anggaran heterosis berdasarkan nilai pertengahan induk dan nilai induk-terbaik yang tinggi dan konsisten bagi hasil dan kebanyakan sifat berkaitan hasil, dengan itu menunjukkan bahawa hibrid-hibrid ini telah memperoleh himpunan gen yang diinginkan yang diperolehi dari induk titisan inbred masing-masing.

Keputusan analisis keupayaan bergabung telah menunjukkan terlibatnya tindakan gen aditif dan bukan-aditif yang ketara dalam pengawalan sifat-sifat yang diukur. Walaubagaimana pun, kesan gen aditif didapati lebih penting dalam pengawalan hasil dan komponen hasil di kalangan hibrid. Antara titisan inbred induk, FTT-1, EE0-2 dan HAW-1 mempunyai kesan GCA positif yang tertinggi dan ketara bagi hasil tongkol segar berkulit di Ladang 2 (masing-masing 1,415.89, 1,132.22 dan 777.89), di Ladang 10 (masing-masing 975.22, 1,321.89 dan 1,204.89) dan pada gabungan kedua-dua lokasi (masing-masing 1,195.56, 1,227.06 dan 991.39). Ini adalah petanda bahawa titisan induk tersebut adalah penggabungan yang terbaik di kalangan titisan inbred induk untuk hasil dan komponen hasil yang tinggi dalam kacukan yang melibatkannya. Antara kacukan yang dihasilkan, P1 × P4 (H3), P4 × P6 (H13), P2 × P4 (H7) dan P5 × P6 (H15) telah dikenalpasti sebagai kombinasi yang terbaik, dengan anggaran SCA positif yang diinginkan bagi sifat yang diukur. Nisbah varians GCA berbanding SCA (GCA/SCA) didapati melebihi 1.0 kebanyakan sifat diukur. Ini telah menunjukkan bahawa tindakan

gen aditif dan gen bukan aditif adalah kedua-duanya penting dalam pengawalan sifat tersebut, walaupun tindakan gen aditif yang lebih menguasai.

Anggaran keboleherisan luas yang tinggi kebanyakan sifat diukur di kedua-dua lokasi dan gabungan antaranya, menunjukkan kecilnya pengaruh persekitaran ke atas fenotip. Anggaran keboleherisan sempit yang tinggi diperolehi kebanyakan sifat diukur di kedua-dua lokasi, menunjukkan kekuatan tindakan gen aditif dalam pewarisan semua sifat tersebut.

Keputusan juga menunjukkan bahawa tiada pertalian antara kesamaan genetik di kalangan enam titisan inbred induk jagung manis tersebut, sebagaimana yang ditunjukkan oleh kesemua penanda DNA SSR yang diamplifikasi, dengan prestasi hibrid, memberi petunjuk bahawa kesamaan genetik berdasarkan penanda SSR yang ditunjukkan dalam kajian ini, tidak boleh menjadi panduan dalam meramalkan prestasi hibrid.

Kesimpulannya, tiap satu dari tiga hibrid terbaik, H11, H12 dan H15 telah dibentuk dari gabungan antara dua dari tiga titisan inbred FTT-1, EE0-2 dan HAW-1. Titis-titis inbred tersebut telah terbukti secara konsistennya menunjukkan kesan GCA yang ketara dan positif bagi hasil dan kebanyakan sifat yang berkait dengan hasil. Oleh itu, hibrid-hibrid yang berpotensi ini perlu diuji selanjutnya dalam pengujian pelbagai-lokasi pada skala yang lebih besar, sebelum ianya diisytiharkan sebagai kultivar baru hibrid jagung manis tropika.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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## LIST OF ABBREVIATIONS

FAO	Food and Agriculture Organization of the United Nations
N	North
E	East
GML	Ground magnesium limestone
Gesaprim 500 FW	1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine
Basta 15	Ammonium 2-amino-4-[hydroxy(methyl)phosphoryl]butanoate
ha	Hectare
ANOVA	Analysis of variance
RCBD	Randomized complete block design
DNMRT	Duncan new multiple range test
MSe	Error mean squares
C.V.	Coefficient of variation
S.D.	Standard deviation
r	Correlation coefficient
$h^2_B$	Broad-sense heritability
$h^2_N$	Narrow-sense heritability
PCR	Polymerase chain reaction
DNA	Deoxyribonucleic acid
Chr.	Chromosome
Bin	A sub-region on corn chromosome
bp	Base pairs
ng	Nanogram

MAS	Marker assisted selection
AFLP	Amplified fragment length polymorphism
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
SSR	Simple sequence repeat
ISSR	Inter simple sequence repeat
RNA	Ribonucleic acid
TBE	Tris-borate/EDTA
Taq	Thermus aquaticus
TE	Tris EDTA buffer
SDS	Sodium dodecyl sulfate
dNTP	2'-Deoxynucleoside 5'- triphosphate
EDTA	Ethylenediaminetetraacetic acid
EtBr	Ethidium bromide
rpm	Revolution per minute
UV	Ultraviolet
v/v	Volume per volume
PPB	Percentage of polymorphic bands
NRM	Number of repeat motifs
$n_a$	Observed number of alleles
$n_e$	Effective number of alleles
$Hom_e$	Expected homozygosity
$Hom_o$	Observed homozygosity
$H_e$	Expected heterozygosity

H <sub>o</sub>	Observed heterozygosity
I	Shannon's information index
Nei's	Nei's expected heterozygosity
PIC	Polymorphic information content
F	inbreeding coefficient
HW	Departure from Hardy-Weinberg linkage equilibrium
NTSYS	Numerical taxonomy multivariate analysis system
PCA	Principle component analysis
PCoA	Principal Coordinate Analysis
UPGMA	Unweighted pair group method using arithmetic averages
S.V.D.	Singular value decomposition
GCA	General combining ability
SCA	Specific combining ability
$\sigma^2$	Variance
$\sigma_G^2$	Genotypic variance
$\sigma_P^2$	Phenotypic variance
$\sigma_{gca}^2 / \sigma_{sca}^2$	ratio of GCA variance to SCA variance
MPH	Mid-parent heterosis
BPH	Better-parent heterosis

## CHAPTER 1

### INTRODUCTION

Sweet corn (*Zea mays* L. *saccharata*) is a specific type of corn, with kernels having high sugar content. It is consumed at the immature stage of the endosperm when the kernels reach the milk stage (Tracy, 2001). In 2016, sweet corn is planted in an area of 1.446 million hectares all around the world with a total production of 13.02 million metric tonnes. The United States is the leading producer (FAOSTAT, 2018).

In Malaysia, sweet corn is grown on 10,042 hectares of land, with a total production of 64,867 metric tonnes in 2016 (FAOSTAT, 2018). It is mainly grown in the states of Johor, Kelantan, Pahang and Perak (MOA, 2016). Sweet corn yield at farm level in Malaysia is 12 metric tonnes per hectare in 2016 (MOA, 2018). Presently, sweet corn cultivation in the country is almost completely dependent on the high quality imported hybrid seeds as planting materials. The locally developed seeds used by the farmers are of low quality. Although the local open-pollinated composite varieties are well-adapted to the growing conditions in Malaysia, they are less preferred because of their inferior taste and low in sweetness. The growers need varieties that are of better quality as in the hybrids. Due to the lack of locally developed hybrid varieties, sweet corn growers purchase imported seeds with a high price, resulting in a high production cost. Therefore, it is highly necessary to increase yield potential of the local sweet corn varieties through breeding programs.

One of the most significant approaches to improve the productivity and quality of sweet corn is by development of local inbred lines that can produce high-yielding hybrid varieties. This process is achieved through successive generations of selfing and subsequent selection of superior lines. The superiority of an inbred line for hybrid production can be estimated using its general and specific combining ability estimates (Falconer, 1981). Molecular markers such as Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphism (SNP) currently play a major role in hybrid corn breeding programs through characterization of genetic variability among inbred lines at the DNA level. Information on genetic variations obtained are used for selection of diverse parental inbred lines for hybrid production.

The study was initiated and carried out with the following hypotheses. The local sweet corn varieties are generally inferior in performance because of their late maturity, having tall plants and are less sweet in taste, but however, are well adapted to the tropical conditions (Saleh et al., 2001; Kashiani *et al.*, 2010). Therefore, breeding efforts are necessary to develop local sweet corn hybrid varieties that are high yielding, having high eating quality and suitable for growing in the local conditions.

The sweet corn breeding program at Universiti Putra Malaysia (UPM) has previously developed several promising populations and inbred lines. The full potential of the locally adapted inbred lines as parents of hybrid sweet corn varieties through selection for their performance and combining ability needs to be fully exploited. Therefore, the present study was undertaken with the following objectives:

1. To evaluate performance and genetic potential of a series of locally developed tropical sweet corn inbred lines,
2. To determine genetic diversity among the inbred lines based on agronomic performance and SSR DNA markers,
3. To evaluate general combining ability and specific combining ability among some selected inbred lines for hybrid production, and
4. To estimate heterosis, heritability and genetic components on the hybrids produced.

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## LIST OF PUBLICATIONS

- Mustafa, N. R., Kashiani, P., Saleh, G. and Abdullah, N. A. P. (2014). *Performance and genetic variation among a series of sweetcorn inbred lines (Zea mays L. saccharata)*. In: Proceeding of the 2<sup>nd</sup> international plant breeding seminar, advanced breeding strategies in crop improvement. 14-15 October, 2014, Agriculture Hall, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia, pp.95.
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- Mustafa, N. R., Saleh, G., Kashiani, P. and Abdullah, N. A. P. (2016). *Combining ability among tropical sweet corn inbred lines and their hybrid performance for agronomic characters*. In: Proceedings of the 7<sup>th</sup> international agriculture congress. Enhancing green agriculture. 4-6<sup>th</sup> October, 2016, Hotel Bangi-Putrajaya, Selangor, Malaysia. pp.90. **(oral presentation)**.
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