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THESIS

GENETIC DISTANCE AND HETEROTIC PATTERN AMONG SINGLE CROSS HYBRIDS IN WAXY CORN

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Waxy corn is a traditional Asian vegetable, consumed as cooked green ears similar to that of sweet corn. A promising hybrid breeding program in Thailand has started, based on germplasm derived from old local landraces, but the genetic diversity and heterotic pattern must be better understood to efficiently plan and use the existing germplasm. The objectives of this study were to; (1) evaluate genetic distance among single cross hybrids of waxy corn in Thailand from different sources (2) investigate correlation among heterotic effects, combining ability and genetic diversity and (3) determine the relationship between phenotypic and genotypic groups investigated by molecular marker. The genetic distance and heterotic pattern were studied using direct diallel crosses of nine hybrids and evaluated in four environments. The result showed that inbreeding depression was high in green ear yield (38%) and marketable dehusk yield (53%), but low in ear length (9%) and ear width (5%). Heterosis of 36 double cross combinations ranged from -33 to 8% for marketable dehusk yield. Troyer's genetic diversity (TGD) estimated from the relationship between heterosis and inbreeding depression varied from 0.40 to 1.16. This indicates that waxy corn hybrids had a diverse genetic background with a good potential for improvement by breeding. The degree of relatedness did not agree with sources of the hybrids. Variation in GCA was significant in green ear yield, marketable dehusk yield and ear size, corresponding variation in SCA was significant for marketable dehusk yield and ear length. Based on cluster analysis of SCA for marketable dehusk yield matrix, two distinct clusters of heterotic pattern were identified for a targeted hybrid breeding. A very high positive correlation (r = 0.98**) was found between TGD and heterosis across four environments. The TGD also positively correlated with SCA (r = 0.63**). Unfortunately, there was no correlation between phenotypic and genotypic groups investigated by molecular marker.

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Kitti Boonlertnirun
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LIST OF ABBREVIATIONS

DC = double cross

EH = ear height

EL = ear length

ENV = environment

EW = ear width

GCA = general combining ability

GY = green yield

H = hybrid parents

IBD = inbreeding depression

LNO = leaf number

LL = leaf length

LW = leaf width

MDY = marketable dehusk yield

NSR = number of seed rows per ear

PCA = principal component analysis

PH = plant height

S = selfed progeny

SEL = seed ear length

SID = silking date

SCA = specific combining ability

SPR1 = leaf greenness in silking stage

SPR3 = leaf greenness at milky stage

STD = stem diameter

TAD = tassel date

TDY = total dehusk yield

TGD = Troyer’s genetic distance

UPGMA = unweighted pair group method using arithmetic average

g_i = general combining ability effect

h_i = variety heterosis

h_{ij} = heterosis

LIST OF ABBREVIATIONS (Continued)

$\hbar$ = average heterosis

s_{ij} = specific heterosis

v_i = variety effect

r = correlation coefficient

GENETIC DISTANCE AND HETEROTIC PATTERN AMONG SINGLE CROSS HYBRIDS IN WAXY CORN

INTRODUCTION

The origin of waxy corn is in southern China. Endosperm of waxy corn producing only the amylopectin is a dull and looks like waxy. This endosperm is controlled by single recessive gene on chromosome 9 and designated as *waxy (wx)* (Fergason, 2001). Waxy corn is popular to consume in Thailand and many countries in Asia because of being sticky, tender and lightly sweet. In Thailand, farmers can grow through the year in irrigated areas. Waxy corns have various kinds and show different characteristics in each location. They can be adapted to local practices. Nowadays, waxy corn hybrids are popular because of its uniformity and seed quality is better than that of local varieties. But, growing the same or similar hybrids in large areas risks to be genetic vulnerability (Troyer *et al*., 1988).

The first step to determine the success of breeding program is to obtain suitable germplasm. The best germplasm should be broad base and high frequency of favorable gene. The local varieties are important germplasm for waxy corn breeding program, but they have narrow genetic base population and high inbreeding depression because they are formed by small population which continuously related within the lines. While commercial single cross hybrids are the other sources of germplasm because line extraction from the population derived from commercial single cross hybrids has been shown to be viable. Populations derived from single cross hybrids are tested in several environments therefore, they are adaptive, highly productive and have a great proportion of fixed favorable alleles (Balestre *et al*., 2008).

The study on genetic diversity of corn helps breeder to plan and efficiently use germplasm for improving a new variety. The single cross hybrid is the aim of corn breeding program because of being utilized from heterosis. The inbred line extraction and selection for specific combining ability are important process to determine the success of corn hybrid breeding. The diallel cross analysis for a fixed set of populations provides a basis for preliminary analysis of heterotic pattern among population crosses. The combining ability, both general combining ability (GCA) and specific combining ability (SCA) are important for understanding the genetic structure of lines and populations. The results are useful for choosing populations and predicting crosses. Heterotic groups and patterns are fundamentally important in hybrid breeding of corn and improve efficiency in breeding program (Reif *et al*., 2003a). Moll *et al.* (1965) studied the relationship between heterosis and the degree of divergence in corn, the result was found that the heterosis increases with increasing of genetic distance were only up to an optimum level. Therefore, accurate assessment of genetic diversity is helpful to assign the lines to be heterotic groups and selects appropriate parental lines for hybrid combinations (George and Regalado, 2004).

Different methodologies are available to investigate genetic diversity such as searching form pedigree data, analysis based on morphological and agronomic traits, but this method is not consistently because corn is open pollinated plant and some phenotypes are varied by genetic and environment interaction. DNA markers are direct method to investigate genetic distance (Smith *et al*., 1997) but it is complex and expensive. Troyer *et al*. (1988) offered a method of measuring genetic diversity between hybrids based on relative heterosis of the hybrid by hybrid cross. Study on genetic diversity of waxy corn in Thailand is necessary for germplasm management. The accurate assessment of genetic diversity is helpful for waxy corn breeding program to maintain the genetic base of elite germplasm and assign the lines to be heterotic groups for selecting appropriate parental lines for hybrid combinations.

OBJECTIVES

1. To evaluate genetic distance among single cross hybrids of waxy corn in Thailand from different sources.

2. To investigate correlation among heterotic effects, combining ability and genetic diversity.

3. To determine the relationship between phenotypic and genotypic groups investigated by molecular marker of waxy corn hybrids.

LITERATURE REVIEW

1. Important characters of waxy corn

Waxy corn (*Zea may* L. *caratina*) was found in China in 1909 (Collins, 1909). The endosperm of waxy corn contains only amylopectin and no amylose in opposition to normal dent corn varieties that contain both. The starch of normal dent corn is characterized by a content of 20 - 36% amylose with the remainder having amylopectin. A single recessive gene located on the short arm of chromosome 9, *wx* codes for the waxy endosperm of the kernel and *Wx* codes for endosperm with normal starch (Neuffer and Coe, 1997).

2. Germplasm of waxy corn in Thailand

Waxy corn production is popular in many locations of Thailand. Farmers can grow throughout the year in irrigated areas. The farmers in each location usually collect seeds from desired corn plants; seeds from the chosen individual plants are bulked and kept to grow for the next cropping seasons. This practice is continuously done, since new corn populations are sometimes established by growing the progeny of a single ear resulted in genetic drift changes in gene frequencies resulted from the creation of small breeding populations. Hence, the local varieties grown in the various countries have adapted to their topographic conditions and farmer's practices, and represent unique sources of genetic diversity. Therefore, there are various types of the local waxy corn varieties in each location. The naming of variety is quite different depending on planting location such as CheingTung, Thein Ayuthaya and Thein Sukhothai, character differences such as SamLee, PumPui, FukBou and HouPee. Wilaiwan *et al*. (2006) collected 34 varieties of waxy corn from different locations and planted to classify their phenotypes. The 30 descriptive phenotypic data showed differently. The kernel color varied in white, yellow and purple. The varieties were classified by ear size and separated to be small and large ear group. The means of ear width and length of small group were 2.5 and 10.5 centimeter respectively and large group were 4.5 and 18 centimeter, respectively. Although local varieties have not been extensively used by breeders because of undesirable agronomic traits, thus they can be served as sources for synthesis of new desirable traits to enhance germplasm performance under abiotic stresses, *i.e.* drought, low soil fertility and acid soils (Beck *et al*., 1997).

The study of phenotypic and genetic diversity to identify groups with similar genetic backgrounds is important for conserving, evaluating and utilizing genetic resources, studying the diversity of pre-breeding and breeding germplasm, and determining the uniqueness and distinctness of phenotypic and genetic constitution of genotypes with the purpose of protecting the breeder's intellectual property rights (Franco *et al*., 2001). Genetic diversity in corn plays an important role for future breeding progress (Reif *et al*., 2003b).

3. Genetic diversity

The importance of germplasm characterization of plant materials has increased due to genetic erosion and habitat destruction by civilized agriculture. Currently, waxy corn hybrids are popular because of high yield and uniformity. More demand of hybrid varieties and similar types of hybrids had led to risk of genetic vulnerability (Troyer *et al*., 1988) and reduction of available genetic variation in germplasm used in breeding programs. The narrow genetic base is problematic in breeding for adaptation to biotic and abiotic stresses. Therefore, study on genetic diversity in hybrids waxy corn improved in Thailand is very important. Because knowledge of the genetic variation and relationship among breeding materials could help to prevent the risk of increasing uniformity in the elite germplasm and also ensure in long-term grain selection (Smith and Smith,1989). The information of genetic diversity will be useful for collection, conservation, selection and hybridization (Beyene *et al*., 2005) and determining the uniqueness and distinctness of the phenotypic and genetic constitution of genotypes with the purpose of protecting the breeder's intellectual property rights (Franco *et al*., 2001).

In corn breeding, the accurate assessment of genetic diversity is helpful to establish and maintain germplasm collection of landrace corn and may guide us in designing strategies maximizing the utility of corn genetic resources (Carvalho *et al.*, 2004), maintaining the genetic base of elite germplasm, assigning lines to heterotic groups and selecting appropriate parental lines for hybrid combinations (Taba *et al*., 1998, Balestre *et al*., 2008). Information about germplasm diversity and relationship among elite materials is fundamentally important in crop improvement (Hallauer and Miranda, 1988). The assessment of genetic diversity has been applied in cultivar identification and studying historical aspects of corn introduction and diffusion in given areas of the world (Sanchez *et al.*, 1993).

Different methodologies are available to investigate genetic diversity including pedigrees based on ancestral relationships, eco-geographic data, agronomic and morphological characteristics, biochemical and molecular data (Kresovich *et al.*, 2005, Dhillon *et al*., 2004, Smith *et al*., 1997). However, the disadvantage of pedigree based on estimation is caused from many assumptions *i.e.* equal genetic contribution on both parents, the unrelatedness of parents with no common ancestral line and no selection, genetic drift and mutation (Kuleung *et al*., 2006). The pedigree information to assess the relatedness of line normally uses in self pollination crops. Accuracy of pedigree is the key for assessment precision. Using pedigree information to assess the relatedness of corn inbred lines is limited because only few generations can be traced back and some inbred lines derived from open pollination population (Jompuk *et al*., 2000).

3.1 Morphological characters for diversity analysis

Genetic diversity analysis based on morphology and agronomic trait and pedigree data has been used for a long time for this purpose; they exhibited the distinct degrees of confidence. But they have been limited by the numbers and influenced by the environments. The key consideration to choose the characters for diversity analysis are least subjected to environmental biases. In addition, for routine characteristics measured quickly and cheaply in the field should be prioritized.

Sanchez *et al*. (1993) studied eleven characters in 30 races of corn from Mexico and concluded that the branched part of the tassel, number of tassel branches, and plant and ear height were the most appropriate traits for classification.

Beyene *et al*. (2005) described genetic relationships in traditional Ethiopian highland corn. Cluster analysis of morphological and marker distances revealed three groups of corn accessions with distinctive genetic profiles and morphological traits. The first group constituted the early maturing and short- statured accessions, which were collected from the Northern agro-ecology from which they probably acquired earliness. The second group included the tall, high yielding varieties, which were currently the most important landraces grown in the Southern and Western parts of Ethiopia. The third group included the tall, late maturing and low yielding accessions, which were being cultivated in some parts of the Northern, Western and Southern highlands of Ethiopia. Despite this limitation, morphological traits were useful for preliminary evaluation because it was fast, simple, and could be used as a general approach for assessing genetic diversity among morphologically distinguishable accessions.

Morphological characteristics are often influenced by the environment. Therefore, they do not express genetic relationships. Morphological data of corn inbred lines cannot be used to determine distinctness because it poorly correlates with pedigree records. While some inbred lines had similar morphologies but different genetic constitution. Besides, these traits revealed differences in terms of genetic distance which were not comprehensible (Smith and Smith, 1989).

3.2 Molecular marker for diversity analysis

DNA-based fingerprinting technologies have been useful in genetic diversity. Data obtained by molecular markers overcome most of the limitations existing in the other kinds of analysis. Characteristics, an almost unlimited number of markers, absence of environmental influence, great number of polymorphic loci, access to contribution of both parents and possibility of comparing genotypes, based on the DNA, make molecular markers very powerful for genetic diversity estimates (Smith, 1988).

Among the molecular markers, microsatellites or SSRs (Simple Sequence Repeats) were chosen as one of the best marker system for genotyping of germplasm collections due to their high performance information content (PIC), codominant inheritance, locus specificity, multi-allelic character, extensive genome coverage and simple detection using labeled primers that flank the microsatellite and hence define the microsatellite locus (Powell *et al.*, 1996). Microsatellites becoming the markers of choice for fingerprinting and genetic diversity studies in plants (Warburton *et al.*, 2002) can be automated and generated large datasets in a short period of time and thus facilitated the evaluation of large numbers of germplasm accessions in seed banks (Rebourg *et al.*, 2001). The chromosomal locations of SSR markers were frequently known, thus providing additional information in genetic diversity SSR markers had the highest expected heterozygosity. Morphological variation did not reflect real genetic variation because of genotype × environment interaction and the largely unknown genetic control of polygenic morphology and agronomic traits (Smith and Smith, 1989). The patterns of divergence revealed by the SSR polymorphisms were consistent with known pedigrees (Senior *et al.*, 1998). SSR profiles can be readily interpreted in terms of alleles at mapped loci across a broad range of corn germplasm. Consequently, SSRs represented the optimum approach for the identification and pedigree validation of corn genotypes compared to other currently available methods (Smith *et al.*, 1997).

In addition, more than 2000 SSRs have already been mapped onto corn chromosomes so that the genome could be uniformly sampled, which increased the precision of genetic diversity estimates and was useful for locating quantitative trait loci (QTL) (Senior *et al*., 1998).

Pejic *et al*. (1998) compared different markers for their effectiveness in estimating genetic similarity among corn inbred lines and found that SSR markers showed the highest level of polymorphism per single marker locus, due to their codominant nature and high number of alleles per locus. In addition, SSR markers could discriminate inbred lines, including those related by pedigree, and were easy to use and automate, relatively inexpensive and mapped to specific genomic location.

Beyene *et al*. (2005) compared among different methods of estimating the genetic diversity. A total of 15 morphological traits, eight AFLP primer combinations and 20 simple sequence repeated (SSR) loci were used. The result found that the mean morphological dissimilarity (0.3 with a range of 0.1 - 0.68) was low in comparison to dissimilarity calculated using SSR markers (0.49 with a range 0.27 - 0.63) and AFLP markers (0.57 with a range 0.32 - 0.69). The correlation between the morphological dissimilarity matrix and the matrices of genetic dissimilarity based on SSR and AFLP markers was 0.43 and 0.39, respectively ($p<0.01$). The correlation between SSRs and AFLPs dissimilarity matrices was 0.67 ($p<0.01$). This congruence indicated that both marker systems were equally suited for genetic diversity study of corn accessions. Cluster analysis of morphological and marker distances showed three groups of corn accessions with distinctive genetic profiles and morphological traits.

4. Heterosis

Heterosis or hybrid vigor, described the superior performance of heterozygous F_1 hybrid plants in terms of increased biomass, size, yield, speed of development, fertility, resistance to disease and to insect pest, or to climatic regions of any kinds compared to the average of their homozygous parental inbred lines. Heterosis was defined as the difference between the hybrid value for one trait and the mean value of two parents for the same trait (Falconer and Mackay, 1996). Sometime heterosis referred to the superiority in performance of the hybrid over either one of its parents (Tollenaar *et al.*, 2004). Currently, heterosis is a major factor for increasing crop production in many crops (Virmani *et al*., 2004).

Two major hypotheses have been proposed regarding the genetic underlying heterosis: (i) dominant hypothesis and (ii) overdominant hypothesis. The dominant hypothesis attributed heterosis to the accumulation of favorable dominant genes or masking of deleterious recessives in the hybrid. The other hypothesis, overdominance argued that the heterozygous combination of the allele at a single locus was superior to either of the homozygous combinations (Tollenaar *et al.*, 2004). Most evidence in corn suggested that genetic basis of heterosis was partial to complete dominance (Hallauer *et al.*, 1988).

The simplest genetically model for estimating heterosis was based on the difference between the hybrid and parental means, which was called midparent heterosis and it was often expressed as a percentage of midparent. Heterosis may be defined as the amount by the mean of F_1 family exceeding its better parent. (Mather and Jinks, 1982) The terms midparent heterosis (MPH) and best parent heterosis (BPH) described the degree of phenotypic difference of a trait in a hybrid (F_1) compared to its parental inbred lines (P_1, P_2). MPH indicated that a trait displayed a hybrid performance was significantly better than the average (midparent) value of the two parental inbred lines ($MPH = F_1 - [(P_1 + P_2)/2]$). BPH, on the other hand, indicated that a hybrid trait performed significantly better than the better (P_b) of the two homozygous parental inbred lines ($BPH = F_1 - P_b$).

Lamkey and Edwards (1999) coined the term panmictic midparent heterosis (PMPH) to describe the deviation in performance between a population cross and the mean of its two parent populations in Hardy-Weinberg equilibrium. Quantitative genetic theory showed the absence of epistasis and two alleles per locus, PMPH was a function of the product of the dominant effect and the square of the difference in gene frequencies at the respective locus (Falconer and Mackay, 1996). Reif *et al.* (2003b) found that PMPH could be expressed as $H_{mp} = y^2d$, where d was the level of dominance and y was the difference in allele frequency of the parents. Therefore, the level of heterosis expressed in hybrid depended largely on allelic frequency differences in two parents and presence of certain level of dominance. Heterosis showed by F_2 population derived from inbreeding F_1 (also called inbred mid parent heterosis) was expressed as $H_{imp} = y^2d$. The differences between panmictic and inbred midparent heterosis reflected the vigor lost due to inbreeding. Genetic divergence and dominance were involved in both inbred and panmictic midparent heterosis (Virmani *et al*., 2004).

The methods predicted heterosis were always measured from diallel cross matting design. Griffing (1956) defined diallel crosses in terms of genotypic values where the sum of general combining abilities for the two gametes was the breeding value of the cross (*i* and *j*). Similarly, specific combining ability represented the dominance deviation value in the simplest case ignoring epistatic deviation. Heterosis and SCA parameters would be correlated and were efficient in the choice of parents or populations (Hallauer and Miranda, 1988) The high correlation among heterosis and SCA observed in 45 double cross hybrids derived from 10 single crosses and that yield was highly correlated with heterosis and SCA (r = 0.75 and 0.82, respectively) (Balestre *et al*., 2008). Mungoma and Pollak (1988) evaluated heterotic combinations among 7 yellow endosperm populations and 3 white endosperm populations; sources of variation for general combining ability (GCA) was significant for root lodging stalk lodging, ear height, days to pollen shade, days to silking, moisture content and yield, but specific combining ability were significant only ear height. Thus, variation among the crosses was mainly due to additive rather than nonadditive effects.

5. Heterotic group

To identify the best inbred combinations for the development of commercial hybrid corn varieties remained the main challenge to corn breeders (Kiula *et al.*, 2007). The expression of heterosis (H) over mid parent depended on the difference in allele frequency (y) of the parents and dominance effect (d) at various loci, that was $H_{mp} = y^2d$ (Falconer and Mackay, 1996). Therefore, genetic diversity was necessary for heterotic expression. Heterotic expression in a cross was a function of genetic background and gene. Heterotic grouping would remain as a key strategic method in management of diverse genetic background and gene (Virmani *et al*., 2004).

Heterotic groups and patterns were fundamental importance in hybrid breeding of corn (*Zea mays* L.) and improved efficiency in breeding program (Reif *et al*., 2003a). The diallel mating design has been largely utilized to identify heterotic patterns when the number of parents was small. Several methods have been proposed for the analysis of a set of *n* parents and their progeny. When data from parents and one set of F_1s were available (reciprocal crosses not included), Griffing's Method 2 (Griffing, 1956), and analyses II and III proposed by Gardner and Eberhart (1966) were suitable (Baker, 1978).

In corn breeding programs, it was necessary to place populations in different heterotic groups. The specific combining ability (SCA) of these populations was a very useful parameter for breeders to choose populations for line extraction to obtain interpopulation hybrids. Knowledge of the effects of the general combining ability (GCA) and SCA helped in testing hypotheses and predicting crosses and importance for understanding the genetic structure of lines and populations. Diallel cross analysis for a fixed set of populations provided a basis for preliminary analysis of heterotic pattern among population crosses. Line crosses derived from different heterotic groups would present superior performance compared to those hybrids formed by crossing the lines of the same group (Hallauer and Miranda, 1988). Several studies have shown that inbred lines from diverse populations tended to be more productive than crosses between inbred lines from the same variety (Dhillon *et al*., 2004).

6. Cluster analysis

Multivariate analysis (*i.e.* discriminant and/or cluster analysis) can classify inbred lines into different groups. This classification may assist in the correct identifications of inbred lines as parents in hybrid breeding studies. Single character evaluation by statistical analysis methods may sometimes cause incomplete and incorrect interpretations. Principal component and discriminant analysis methods can be used for the combined analysis and provide more reliable conclusions in identification of genetic materials. Aydın *et al.* (2007) determined the genetic variation between dent corn inbred lines from diverse background and topcrosses created by crossing each inbred line with the tester line ‘FrMo 17’, by using multivariate analysis. Discriminant analysis used for revealing general distances between genotypes as numerical values (D2) indicated which traits could be used to group genotypic differences. Based on the D2 values obtained from discriminant analysis, the most different lines were number 17 (Pool 30) and number 31 (Fr Mo 17) while the most similar ones were number 3 (B 87) and number 18 (Pool 30a). The data pointed out that the both genetic distance and combination of good parental genes were responsible for heterosis and desirable F_1’s traits.

Cluster analysis aims to group items. In this case, genotypes based on the characteristics that they posse so that individuals with similar descriptions are mathematically gathered into the same cluster. Clustering methods usual lead to a graphical representation such as tree or dendrogram in which clusters may be visually identified (Mohammadi and Prasanna, 2003).

7. Genetic distance and heterosis association

Heterosis increases with increasing genetic distance were only up to an optimum level (Moll *et al.*, 1965). Genetic diversity and heterosis are positive association. Hybrids of diverse material yield more than hybrids of related materials (Troyer *et al.*, 1988). Amorim *et al.* (2006) found high correlation between grain yield and genetic divergence for interpopulation hybrids (r = 0.84), but this correlation was low for intrapopulation hybrids.

Benchimol *et al*. (2000) investigated genetic distances among tropical corn materials and their relationship to heterotic group allocation and hybrid performance. It found that genetic distances (GDs) were greater average for BR-105 × BR-106 lines (0.77) than for BR-106 × BR-106 (0.71) and for BR-105 × BR-105 (0.69) lines. Cluster analysis resulted in a clear separation of BR-105 and BR-106 population was according to pedigree information. Correlations of parental GDs with single crosses and their heterosis for grain yield were high for line crosses from the same heterotic group and low for line combinations from different heterotic groups. Their results suggested that RFLP-based GDs was efficient and reliable to assess and allocate genotypes from tropical corn populations into heterotic groups. However, RFLP-based GDs was not suitable for predicting the performance of line crossed from genetically different heterotic groups.

Reif *et al*. (2003b) investigated the relationship between heterosis and genetic distance determined with simple sequence repeat (SSR) markers in seven tropical corn populations and concluded that SSR markers provided a powerful tool for grouping germplasm and had a valuable complementation to field trials for identifying groups with satisfactory heterotic responses. The analysis of molecular variance (AMOVA) revealed that 89.8% of the variation was found within populations and only 10.2% between populations. The correlation between PMPH and the squared modified Roger's distance (MRD) based on SSR markers was significantly positive (p<0.05) only for grain yield (r = 0.63).

Kiula *et al.* (2007) reported that better F_1 hybrid performance predictions can be achieved by integrating molecular and F_1 phenotypic data. They investigated DNA fingerprint of 21 inbred lines using amplified fragment length polymorphism markers. Parents and 210 F_1 progenies were evaluated in the field. Joint data analysis mostly revealed a tighter association between GD and the F_1 performance or mid parent heterosis in the intergroup than in the intragroup crosses. Despite these correlations, intergroup crosses should always be field-tested before their release. Crosses showing low GD values should be discarded to avoid field-testing costs.

Lanza *et al.* (1997) evaluated the genetic diversity of 18 corn inbred lines, and determined the correlation between genetic distance and single-cross hybrid performance. They have used random amplified polymorphic DNA (RAPD), a PCR-based technique. Eight of these lines came from a Thai synthetic population (BR-105), and the others derived from a Brazilian composite population (BR-106). The genetic distances (GD) were correlated with important agronomic traits for single-cross hybrids and heterosis. No correlation was found when group division was not considered, but significant correlations were detected between GI×GII and GI×GIII GDs with their respective single-cross hybrid grain yield values. Three groups were identified; that is, lines within the BR-106 population were divided into different groups and the BR-105 population remainders were mostly in one group. The results indicated that RAPD can be used as a tool for determining the extent of genetic diversity among tropical corn inbred lines, allocating genotypes into different groups, and also aiding in the choice of the superior crosses to be made among corn inbred lines, and reducing the number of required crosses under field evaluation.

Choukan *et al*. (2006) studied the level of genetic diversity and relationships among the most commonly used, medium to late maturing Iranian corn inbred lines, and suggested that heterotic groups among the lines using genetic distance measured by the SSR markers showed the highest distance between the cluster of Reid Yellow Dent related lines and the cluster of Lancaster Sure Crop related lines, and this pattern has produced some of the highest yielding hybrids in Iran.

Amorim *et al*. (2006) worked with three S_0 populations derived from different single cross hybrids and selected the two most and the two least productive inter- and intrapopulation hybrids from a total of 163 assessed hybrids. The 48 parent lines of these hybrids were genotyped with 47 simple sequence repeat (SSR) primers. These authors obtained high correlation between grain yield and genetic divergence for interpopulation hybrids (r = 0.84), but this correlation was low for intrapopulation hybrids, indicating that the markers would be efficient in predicting hybrids derived from different heterotic groups.

8. Genetic diversity among single cross hybrids

Single cross hybrids were accepted in corn production. The replacement of open varieties with single cross hybrids increases yield of corn in the last five decades. Farmers prefer hybrids due to their good performances in varying weather condition; they can still produce higher yield. Both farmers and seed companies took adventage from superior, widely adapted hybrids. Popularity, spread and similarity of hybrid cause a risk of genetic vulnerability (Troyer *et al.*, 1988). However, single cross hybrids are interesting germplasm for inbred improvement. Line extraction from populations derived from commercial single cross hybrids has been shown to be viable. The advantage of this technique compared to lines obtained from open pollination varietal lines mainly derived from single cross hybrids are tested in several environments; they are adapted, highly productive and have a great proportion of fixed favorable alleles (Balestre *et al*., 2008). Genetic diversity of single cross hybrids was frequently answered questions about the risks of genetic vulnerability and potential of these materials for inbred line extraction.

Troyer *et al.* (1988) offered a straightforward method of measuring genetic diversity between hybrids based on relative heterosis of the hybrid by hybrid cross. Their method equated genetic diversity with degree of the heterozygosity using changes in heterosis as a measure. The method assumed that hetrosis caused by some degree of dominance and epistasis was absent. Comparisons between the hybrid-by-hybrid cross and the mean performance of the two hybrids were made to determine if the two hybrids were identical (0.0 GD) or unrelated (1.0 GD). Performance of the hybrid-by-hybrid cross was quantified by the inverse relationship of GD to inbreeding depression. Data were collected for grain yield, growing degree units to pollen shed, harvested grain moisture and plant height, giving the best spread of estimates for GD.

Jompuk *et al.* (2000) measured genetic diversity of twelve commercial hybrids in Thailand by Troyer's genetic distance method. The genetic diversity index of each pair of hybrids ranged from 0.25 - 0.98 and averaged of 0.68, GD of hybrids from the same company ranged from 0.25 - 0.67, while the another company ranged from 0.37 - 0.98. The results showed that the hybrids in Thai market still had considerable genetic diversity with noticeably exchanged of genetic background.

Phumichai *et al.* (2008) evaluated selected commercial hybrid corn varieties which were used for further improvement of germplasm, and to compare the observed grain yield of single-cross hybrids made from these hybrids with GD between parental varieties using SSR-markers, and their selfed and hybrid (double-cross hybrid) progenies grown in six locations in Thailand. Troyer's genetic distance (TGD) calculated from yield data, varied from 0.493 to 1.015 between different parental combinations. A significant positive correlation was found between the GD and TGD across six locations (0.66**). The GD was positively correlated with specific combining ability (SCA) as well across all locations (r = 0.76**). The highest yielding hybrids (KU4452×PAC220) had a lower SCA value but more genetic distance between the parents, while the hybrids DK888×PIO87and BIG919×NS-2 showed the 1st and 2nd highest values of SCA and relatively high yield, respectively. Therefore, based on the combined criteria with SSR-based GD, TGD, grain yield and GCA, they suggest that farmers could produce their own hybrid populations (double-cross hybrids) [(KU4452×PAC220), (NK48×PAC220), (KU4452×NK48) and (PAC220×PIO87)] using four recommended commercial varieties (single-cross hybrids) (PIO87, KU4452, NK48 and PAC220). Breeders could choose these four varieties to develop their own inbred lines. Furthermore, breeders in Thailand could begin three breeding populations corresponding to the three distinct clusters, to use for recurrent reciprocal selection and the eventual creation of new inbred lines crossed between groups, should create superior hybrids for our future breeding efforts.

Balestre *et al*. (2008) studied the relationship between the genetic distances (GD) based on SSRs markers of single cross hybrids with yield, heterosis and specific combining ability (SCA) in the double cross hybrid synthesis. The average GD of the 10 single cross hybrids was 0.84. This relatively high distance was due to the use of hybrids from four different companies. The greatest GDs were detected in the 4 × 9 (0.95) and 1 × 6 (0.95) hybrids, both had high yields. It was also observed that hybrids with smaller GD (0.65) produced a low yield double cross hybrid (1 × 4). These hybrids were derived from the same company and probably had parents in common or very closely in their genetic constitution. There was a medium correlation between GD and heterosis (r = 0.40) and GD and SCA (r = 0.38). The intergroup hybrids placed by genetic grouping were generally more productive than intragroup hybrids, and the hybrids with GD greater than 0.84 had the maximum heterosis and SCA. It was concluded that the SSRs markers were efficient in identifying the greater value of heterosis and SCA, and placing the parents to heterotic groups.

Souza *et al.* (2001) assessed the genetic divergence of some single cross hybrids and evaluated their potential for inbred line extraction in Brazillian corn breeding programs. The genetic divergence of single cross hybrids averaged of 0.65 indicated that the single cross hybrids were divergent and the risk of genetic vulnerability was small. The hybrids differed in the potential for inbred lines extraction.

MATERIALS AND METHODS

Plant materials

Nine waxy corn hybrids were obtained from different sources as follows: NSW, TSW and GR484 from Bangkok Seed Industry Company, KWSX91 from Kasetsart University, KKU1116 and KKU2901 from Khon Kaen University, BW852 from East West Seed Company, WPP004 from JiaTai Seed Company and Dr. PEK from Sweet Seed Company. They were grown and crossed during December 2007 to February 2008 in a half diallel mating manner to produce thirty-six double crosses. The original F_1 hybrids were also self-pollinated resulted in nine selfed progenies. The seeds of original F_1 hybrids or nine hybrid parents (H), thirty six double crosses (DCs) and nine selfed progenies (S) were used as the entries materials in yield testing.

Methods

1. Field evaluations

The experiment was conducted in two locations, each in two seasons. The first location was Suwan Farm (National Corn and Sorghum Research Center) Nakhon Ratchasima province, the northeastern region of Thailand at the latitude of $14^{o}30'$N and longitude of $101^{o}0'$E, in rainy season (August-September, 2008) and dry season (December, 2008 – January, 2009). The soil type was Rhodic Kandiustox. The second location was Agronomy Farm, Faculty of Agricultural Technology and Agro-industry, Rajamangala University of Technology Suvarnabhumi, Phranakhon Si Ayutthaya province, the central region of Thailand, at the latitude of $14^{o}36'$N and longitude of $100^{o}60'$E, in dry season (December, 2008 – January, 2009) and early rainy season (May-June, 2009). The soil type of this location was Vertic Endoaquept. The experimental design in each environment was a randomized complete block with two replications. Each plot consisted two rows, 5 m long with 0.25×0.75 m^2 of plant row spacing, resulting in 5.3 plants/ m^2. Weeds were controlled by spraying atrazine at 2.3

l/ha as pre-emergence, and hand weeding thereafter. Fertilizer was basally applied with 46.8 kg/ha each of N, P_2O_5 and K_2O**,** which was provided by compound fertilizer, formula 15-15-15, at the rate of 312.5 kg/ha and top dressed with urea at the rate of 312.5 kg/ha (143.8 kg/ha of N) at 20 days after emergence**.**

2. Data collection

Performances of nine waxy corn hybrid varieties were collected for group classification. They were green yield (GY), maketable dehusk yield (MDY), total dehusk yield (TDY), ear length (EL), seed ear length (SEL), ear width (EW), number of seed rows per ear (NSR), plant height (PH), ear height (EH), leaf greenness in silking stage (SPR1), leaf greenness at milky stage (SPR3) measured by chlorophyll meter (SPAD 502), tassel date (TAD), silking date (SID), stem diameter (STD), leaf number (LNO), leaf length (LL) and leaf width (LW).

3. Data analysis

Performances of nine waxy corn hybrid varieties were classified by 17 traits of yield and morphology. Multivariate techniques were applied to create new sets of variables to characterize performance of hybrid varieties. The standardized trait means of hybrid varieties were used to perform principal component scores utilizing for clustering hybrid varieties. The first three principal component scores were plotted to visually locate for grouping the hybrid varieties. Phenotypic dissimilarity (PD) among the parents was estimated from Euclidian distance matrix. Each distance was estimated by the formula as follow: $D_{rs} = [\sum(X_{rj}-X_{sj})^2]^{1/2}$ where X_{rj} and X_{sj} are the r^{th}, and s^{th} entries measured on j^{th} trait. Once the distance matrix was determined, a dendrogram was constructed based on unweighted pair group method using arithmetic average (UPGMA).

Analysis of variance was performed for each trait and combined across four environments. The entries were recorded on green yield (GY), marketable dehusk yield (MDY), ear size (ear length (EL), ear width (EW), number of seed rows per ear (NSR)) and agronomic traits (plant height (PH), ear height (EH), tassel date (TAD), and silking date (SID)). Variation among entries was partitioned into non-orthogonal contrast between hybrids (H) and selfed-progenies (S) (df = 1), the variation among H, S (each with df = 8) and hybrid cross or double cross (DCs) (df = 35). Statistic analysis was performed by R (R Core Team, 2012).

The analysis II and analysis III of Gardner and Eberhart (1966) were employed to estimate genetic information of the hybrid parents and their crosses.

The analysis II was based on fitting the hybrid (H) and hybrid cross (DC) means to the linear model:

$$Y_{ij} = \mu_v + ½\,(v_i + v_j) + \gamma(\bar{h} + h_i + h_j + s_{ij})$$

where Y_{ij} is trait mean of an entry; μ_v is the overall mean of the trait; v_i and v_j are estimates of the varietal effects for parental hybrid i and j, respectively; $\bar{h}$ is the average heterosis contributed by the DC; h_i and h_j are heterotic effects for hybrids i and j, respectively; and s_{ij} is the specific heterosis that occurs when hybrid i is mated to hybrid j; $\gamma = 0$ when $i = j$ (*i.e.* H) and $\gamma = 1$ when $i \neq j$ (*i.e.* DC).

The analysis III was performed based on fitting the hybrid cross (DC) means:

$$Y_{ij} = \mu_c + g_i + g_j + s_{ij}$$

where Y_{ij} is the mean of the cross between hybrid i and j; μ_c is the overall mean of the DC; g_i and g_j are general combining ability effects and s_{ij} is specific combining ability effect of the hybrid parents.

Environments and replications within environment were considered as random effects while the other sources of variation were considered fixed effects. Genotype ×

environment interaction was used as the denominator for testing significance among the entries. UPGMA dendrogram based on SCA matrix was performed to classify heterotic pattern among nine waxy hybrid varieties using R (R Core Team, 2012).

Inbreeding depression (IBD) was determined from the equation, $\mathrm{IBD} = \frac{H-S}{H}$, while mid-parent heterosis (MPH) was determined from the equation, $\mathrm{MPH} = \frac{DC-MP}{MP}$. Where H is the trait average across four experiments of the hybrid varieties; S is the trait average of selfed progeny of the hybrids, DC is the trait average of the double cross combinations, $\mathrm{MP} = \frac{P_1 - P_2}{2}$ in which P_1 and P_2 are the trait average of the hybrid parents.

Troyer's genetic distance (TGD) between hybrid parents in each replication within environments was calculated from the equation, $\mathrm{TGD} = 1 - \frac{H-DC}{H-S}$. Where H is the trait means of the two hybrid parents, DC is the double cross trait mean and S is the trait mean of the selfed progenies (Troyer *et al*., 1988). Analysis of variance for TGD was performed across environments.

The relationship among GD, H, C, and S is graphically shown in Figure 1

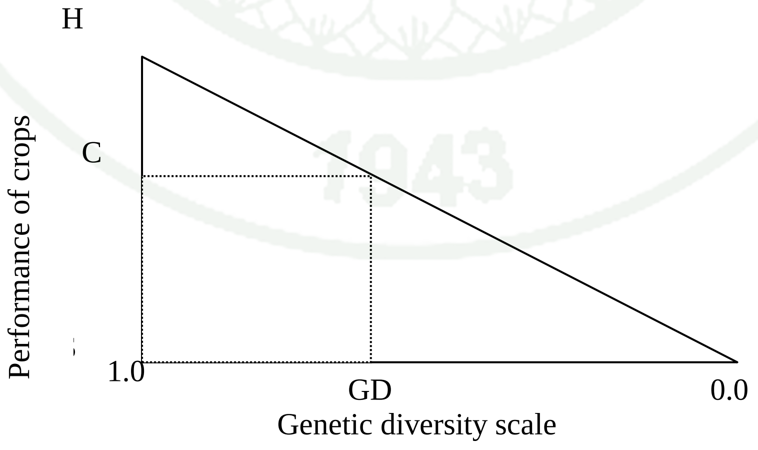

Figure 1 Algebraic formula for determining genetic diversity with geometric interpretation for use with yield or possibly other performance data.

The Troyer’s genetic distance can be interpreted to relate between two hybrids. The value of hybrid-by-hybrid cross is higher than performance cross average of two hybrids (less inbreeding depression) indicates more genetic diversity between the hybrids while the lower performance cross average (more inbreeding depression) indicates less genetic diversity. Two identical hybrids have an expected GD of 0 when (H - C) = (H - S). Two closely related hybrids with GD of 0.25 probably are related on both sides of the pedigree. Two hybrids with one inbred in common and the other unrelated inbred lines (A×B and A×C) had an expected GD of 0.5 because of the specific combining ability of the unrelated inbred lines (B×C). Two slightly related hybrids with a GD of *ca.* 0.75 probably had unrelated inbred lines on one side of the pedigree and a distantly related (cousin) inbred on the other. Two unrelated hybrids had an expected GD of 1.0 (H-C) = 0.

4. **Estimating genetic distance base on simple sequence repeat**

4.1 DNA extraction

DNA from each leaf of 9 single cross hybrids was isolated and extracted using a modified CTAB procedure according to the CIMMYT protocol for corn genotyping using SSR markers (George and Regalado, 2004).

4.2 Microsatellite markers

Twenty SSR markers (two markers per chromosome) distributed throughout the corn genome were chosen from MaizeGDB based on their repeated unit, base composition and localization on chromosomal regions involved in agronomic traits were previously reported. The SSRs used in this study are listed in Table 1. In addition, primer sequences were available from MaizeGDB (www.maizegdb.org).

Table 1 Primer sequences of SSR marker

SSRs marker	Repeat	bin	SSR marker	Repeat	bin
bnlg1007.	AG(15)	1.02	umc1725		1.11
p-bnlg1092	(AG)30	2.01	p-umc1126	ACC	2.08
p-umc1394	(AT)10	3.01	p-bnlg1754	AG(20)	3.09
p-bnlg1370	AG(37)	4.00	p-bnlg1337	AG(21)	4.11
p-bnlg565		5.02	p-umc1225	(AG)6	5.08
p-bnlg249		6.01	p-umc1653	(GAAA)24	6.07
p-umc1426	(AGAGG)4	7.0	p-umc1671	(AGC)7	7.05
Umc1359		8.00	p-umc1069	(GGAGA)6	8.08
p-phi033	AAG	9.01	p-umc1137	(CT)15	9.08
p-phi041	AGCC	10.00	p-bnlg1450	AG(34)	10.07

4.3 Amplification

PCR reactions were performed in a volume of 10 µl each containing 1× PCR buffer. The components of PCR used in this study are in the Table 2.

Table 2 The components of PCR in a volume of 10 µl

Stock	Final concentration	Volume per reaction (ul)
Sterile ultrapure water	-	5.6
10X PCR buffer 1X	-	1.0
25 mM MgCl2	2.0 mM	0.8
10 mM dNTP	0.25 mM@	1.0
5 uM @ Primer Mix (F and R)	0.25 mM @	0.5
5U/ul Taq DNA Polymerase	0.5 U	0.1
Total reaction volume		10.0

The amplification reaction consists a denaturing step of 2 min at 94 ºC, followed by 30 cycles with 94 ºC for 2 min, annealing reaction of 1 min at 56 ºC and terminate at 72 ºC for 1 min.

4.4 Gel electrophoresis

Obtained PCR product was added with 3 ml loading buffer (10 mM NaOH, 95% formamide, 0.05% bromophenol blue, 0.5% xylene cyanol FF) and electrophoresis on 1%agarose gel in 1XTAE buffer (pH 8.3) at a constant voltage (3.00 kV) for 0.5 h and detected by ethidium bromide staining, visualized and photographed polymorphism under UV fluorescence (CIMMYT, 2006)

4.5 Genetic distance analysis

SSR polymorphism was scored on presence (1) or absence (0) of DNA band basis. The two varieties and presence- absence of DNA band data was possible to be four types of match as Table 3.

Table 3 Four types of match of possible presence-absence of DNA band basis

Possible match		Variety$_j$	
		present	absent
Variety$_i$	present	1-1 (a)	1-0 (b)
	absent	0-1 (c)	0-0 (d)

To pair off genetic distance of all possible matches was estimated by Euclidean distance:

$$\text{distance } (ij) = \sqrt{b + c}$$

To pair off genetic similarity of all possible matches was estimated by Jaccard coefficient as follow:

Jaccard similarity (*ij*) = a/(a+b+c+d)

5. Relationship among genetic distance, heterosis and combining ability

Pearson correlation coefficients (r_{xy}) determined from relationships among DC means across four environments, specific combining ability, heterosis and Troyer’s genetic distance indicated relationship between genetic distance and heterosis.

$$r_{xy} = \frac{\sum XY - \frac{\sum X \sum Y}{n}}{\sqrt{\left(\sum X^2 - \frac{(\sum X)^2}{n}\right)\left(\sum Y^2 - \frac{(\sum Y)^2}{n}\right)}}$$

RESULTS AND DISCUSSION

1. Performance of hybrids and inbreeding depression

The combined analysis of variance showed highly significant difference ($p<0.01$) among 54 entries (9 H, 9 S and 36 DC) for all traits. Similarly, the entry × environment interaction was also significant for all traits.

1.1 Yield and ear size

Yields of tested hybrid varieties were significantly different, PEK gave the highest green yield and marketable dehusk yield of 14.51 and 9.49 t/ha respectively (Table 4). The comparison of H versus S, provided a measure for an average IBD, was significant ($p<0.01$) for yield. Yield of all progenies produced from hybrid self significantly decreased. The decreases of green yield and marketable dehusk yield were between 30.4 to 48.3% and 44.3 to 61.2%, respectively. Both green yield and marketable dehusk yield of BW and WPP seriously expressed inbreeding depression. Interaction between H vs S and environment was not significant for yield; it meant that environment similarly affected on inbreeding depression of hybrids (Table 4).

The comparison of H versus S on ear size, significant difference ($p<0.01$) in terms of ear length and ear width were found. Ear size of S lines was decreased and smaller than that of H average by 9.5% for ear length and 4.7% for ear width indicating inbreeding depression. When GR484, the hybrid variety with the longest ear of 18.2 cm was selfed, ear length of its progeny was decreased by 15.6% whereas WPP, the hybrid variety with the widest ear of 5.4 cm was selfed, ear width of its progeny was remarkably decreased by 11.2 %. Ear length and ear width of NSW, were not significant between H and S (Table 4).

Inbreeding depression in marketable dehusk yield was more pronounced than the other traits. Similar results were reported by Saleh *et al.* (1993) who found that IBD in sweet corn populations caused yield loss with the estimated IBD 33.58%

for GY, 36.47% for MDY, 20.48% for EL, and 8.73 for EW. Inbreeding depression in the F_2 may be due to the accumulation of recessive alleles in homozygous state.

1.2 Agronomic characteristics

Hybrid varieties showed significant differences in terms of plant height (PH), tassel date (TAD) and sillking date (SID), except ear height (EH). To compare hybrid variety (H) and its selfed line (S), only plant height was significant (Table 5). GR484, the hybrid variety, was the highest plant height of 163 cm whereas the selfed line with 139 cm height showed the highest inbreeding depression of 14.2%. Inbreeding depression values of WPP, PEK and KWSX varieties for plant height were 13.5, 12.0 and 10.2 %, respectively while the rest varieties were not different. Regarding with tassel date and silking date, there were no significant difference between hybrid and its self lines (Table 5).

2. Varietal cluster based on morphological traits and yield

2.1 Principal component analysis

Seventeen agronomic traits of 9 waxy corn hybrids were studied. Correlation coefficients between variables were significant. It was found that some traits were also related, *i.e.* green yield positively related with marketable dehusk yield, number of seed rows positively and negatively related with ear width and ear length, respectively and plant height positively related with ear height and leaf length (Table 6)

Table 4 Mean performances of nine parental waxy corn hybrids (H), self (S) and inbreeding depression (%IB) for yield and ear size.

Variety	GY[1/] (t/ha)			MDY (t/ha)			EL (cm)			EW (cm)			NSR (row)		
	H	**S**	**%IB**	**H**	**S**	**%IB**	**H**	**S**	**%IB**	**H**	**S**	**%IB**	**H**	**S**	**%IB**
NSW	12.13	8.10	33.2**[2/]	7.93	4.15	47.6**	16.6	15.9	4.7^{ns}	4.0	4.0	-0.6^{ns}	13.2	12.9	2.3^{ns}
BW	13.39	6.93	48.3**	8.40	3.28	61.0**	15.9	14.6	8.2**	4.3	4.0	6.7*	12.6	11.9	5.9^{ns}
WPP	12.48	7.13	42.9**	8.63	3.35	61.2**	13.3	11.9	10.0**	5.4	4.8	11.2**	16.5	15.0	9.1**
PEK	14.51	9.64	33.6**	9.49	4.90	48.4**	17.9	16.3	8.6**	4.1	3.9	4.3^{ns}	12.9	12.8	0.4^{ns}
GR484	12.79	7.56	40.9**	8.74	3.69	57.8**	18.2	15.3	15.6**	4.1	3.8	8.2**	12.9	12.6	2.5^{ns}
KWSX91	11.46	7.65	33.3**	6.43	3.39	47.3**	16.5	14.8	10.5**	4.3	4.1	3.3^{ns}	13.3	12.7	4.1^{ns}
KKU1116	12.44	7.94	36.2**	8.24	3.69	55.2**	17.4	15.2	12.4**	4.1	3.9	4.5^{ns}	14.1	13.5	4.3^{ns}
KKU2901	13.03	7.96	38.9**	7.94	3.60	54.6**	16.5	15.1	8.6**	3.9	3.8	2.8^{ns}	12.5	12.4	0.8^{ns}
TSW	10.45	7.28	30.4**	6.66	3.71	44.3**	14.5	13.5	7.1*	3.5	3.5	1.8^{ns}	13.2	14.2	-7.6*
mean	12.52	7.8	37.5	8.05	3.75	53	16.3	14.8	9.5	4.2	4.0	4.7	13.5	13.1	2.4
LSD$_{.05}$		1.79			1.25			0.90			0.23			0.87	
LSD$_{.01}$		2.36			1.61			1.19			0.32			1.15	
							F-test								
H		*			*			**			**			**	
S		*			ns			**			**			**	
HvsS		**			**			**			**			**	
Env × H		**			**			*			*			ns	
Env. × S		ns			ns			*			**			ns	
Env. × H vs S		ns			ns			ns			ns			ns	

[1/] GY= Green yield, MDY = maketable dehusk yield, EL = ear length, EW = ear width, and NSR = number of seed rows per ear

[2/] ^{ns} = not significant differentce * and ** = Significant at 0.05 and 0.01 probability level, respectively.

Table 5 Mean performances of nine parental waxy corn hybrids (H), self (S) and inbreeding depression (%IB) for agronomic traits.

Variety	**PH[1/] (cm)**			**EH (cm)**			**TAD (day)**			**SID (day)**		
	H	**S**	**%IB**	**H**	**S**	**%IB**	**H**	**S**	**%IB**	**H**	**S**	**%IB**
NSW	152.4	147.5	3.2^{ns} [2/]	68.3	68.6	-0.5^{ns}	48.1	48.5	-0.8^{ns}	49.5	50.3	-1.5^{ns}
BW	131.1	122.8	6.4^{ns}	63.1	57.0	9.7^{ns}	50.1	50.3	-0.4^{ns}	52.1	52.4	-0.5^{ns}
WPP	123.5	106.9	13.5*	61.9	51.3	17.2^{ns}	46.9	47.6	-1.5^{ns}	48.1	49.1	-2.1^{ns}
PEK	149.5	131.6	12.0**	75.6	66.9	11.6^{ns}	47.4	47.5	-0.2^{ns}	49.0	49.9	-1.8^{ns}
GR484	163.0	139.9	14.2**	77.9	67.0	14.0^{ns}	48.3	48.3	0.0^{ns}	49.9	50.3	-0.8^{ns}
KWSX91	151.8	136.3	10.2*	74.5	67.0	10.1^{ns}	48.1	47.5	1.2^{ns}	49.6	50.1	-1.0^{ns}
KKU1116	124.0	114.4	7.8^{ns}	59.5	53.8	9.7^{ns}	46.4	46.9	-1.1^{ns}	48.5	49.0	-1.0^{ns}
KKU2901	122.9	113.5	7.6^{ns}	67.0	58.1	13.2^{ns}	46.9	48.3	-3.0^{ns}	48.9	50.4	-3.1^{ns}
TSW	143.0	140.3	1.9^{ns}	71.1	72.8	-2.3^{ns}	47.8	48.5	-1.5^{ns}	49.1	51.0	-3.8^{ns}
Mean	140.1	128.1	8.6	68.8	62.5	9.2	47.8	48.1	-0.6	49.4	50.3	-1.7
LSD.05		12.66			9.21			1.41			1.57	
LSD.01		16.72			12.16			1.86			2.08	
					F-test							
H		**			ns			*			*	
S		**			**			*			ns	
HvsS		*			ns			ns			ns	
Env x H		**			**			**			**	
Env. x S		Ns			ns			**			**	
Env. x H vs S		Ns			ns			ns			ns	

[1/] PH = plant height, EH = ear height, TAD = tassel date, and SID = silking date

[2/] ^{ns} = not significant differentce * and ** = Significant at 0.05 and 0.01 probability level, respectively.

Table 6 Correlation coefficient among 17 traits of 9 waxy corn hybrids

	TDY[1/]	MDY	EL	SEL	EW	SRN	PH	EH	SPR1	SPR3	TAD	SID	STD	LNO	LL	LW
GY	0.88** [2/]	0.88**	0.47	0.53	0.24	-0.16	-0.09	0.01	0.45	0.47	0.06	0.18	0.26	0.10	-0.05	0.64*
TDY		0.95**	0.23	0.25	0.54	0.21	-0.01	-0.03	0.23	0.37	0.06	0.08	0.14	0.28	0.22	0.33
MDY			0.32	0.37	0.36	0.12	-0.08	-0.08	0.27	0.41	-0.08	-0.01	0.13	0.09	0.07	0.52
EL				0.97**	-0.45	-0.65*	0.48	0.45	0.59*	0.23	0.05	0.19	-0.01	-0.22	0.10	0.64*
SEL					-0.52	-0.75**	0.44	0.44	0.50	0.19	0.16	0.32	0.03	-0.26	0.11	0.65*
EW						0.83**	-0.36	-0.40	0.05	0.38	-0.13	-0.20	0.24	0.26	0.12	-0.27
SRN							-0.41	-0.49	-0.12	0.20	-0.45	-0.56	0.04	0.20	-0.06	-0.34
PH								0.87**	0.00	-0.30	0.35	0.22	-0.26	0.43	0.78**	-0.13
EH									0.24	0.00	0.19	0.09	0.07	0.52	0.62*	0.11
SPR1										0.84**	-0.37	-0.22	0.63*	-0.02	-0.22	0.67*
SPR3											-0.45	-0.32	0.79**	-0.09	-0.21	0.56
TAD												0.96**	-0.01	0.08	0.55	-0.49
SID													0.09	-0.13	0.38	-0.30
STD														-0.01	-0.06	0.20
LNO															0.42	-0.19
LL																-0.47

[1/] GY = green yield, MDY = maketable dehusk yield, TDY = total dehusk yield, EL = ear length, SEL = seed ear length, EW = ear width, NSR = number of seed rows per ear, PH = plant height, EH = ear height, SPR1 = leaf greenness in silking stage, SPR3 = leaf greenness at milky stage, TAD = tassel date, SID = silking date, STD = stem diameter, LNO = leaf number, LL = leaf length, and LW = leaf width.

[2/] * and ** = significant difference at the 0.05 and 0.01 probability, respectively.

When some variables were reduced by combining related variables to be principal component, but variations of total variables were still conserved and found that 4 components having eigenvalues equal to or greater than two (≥2.0) were retained as meaningful and worthy of interpretation. These 4 components accounted for 84.30% of the total variation in the data set (Table 7). The First Principal Component (PC1) had an eigenvalue of 4.97 and explained 29.21% of the total variation in the data set (Table 7). Four variables involving ear size (EL, SEL, EW and SRN) weighted higher than the other variables in PC1, this suggested that PC1 represented the equivalent of four individual variables and could reflect ear size. Eigenvalues of PC2, PC3 and PC4 were 4.42, 2.88 and 2.06, respectively. Loaded variables in PC2 and PC3 could reflect yields (GY, TDY and MDY) and plant structure (PH, EH, LNO and LL), respectively. While flowering dates (TAD, SID) were loaded in PC4, the factor contributing to PC1 was ear size (29.21%). While PC2, PC3 and PC4 attributed to yield (26.01%), plant size (16.97%), and flowering date (12.11%), respectively.

Table 7 Eigenvalues and their contribution to total variation extracted by Principal Component Analysis (PCA).

Trait	**PC1**	**PC2**	**PC3**	**PC4**
	-------------------Component score coefficient-----------------			
GY[1/]	.036	**.264**	-.047	.056
MDY	-.003	**.313**	-.014	-.019
TDY	-.057	**.316**	.037	.028
EL	**.207**	.057	.023	-.039
SEL	**.214**	.070	-.012	.019
ED	**-.226**	.136	.041	.040
SRN	**-.213**	.063	.049	-.120
PH	.070	-.020	**.296**	-.055
EH	.070	-.086	**.315**	-.076
SPR1	.061	-.056	.050	-.063
SPR3	-.035	-.021	.012	-.019
TAD	-.021	.022	-.017	**.378**
SID	.021	.023	-.099	**.407**
STD	-.081	-.129	.000	.191
LNO	-.114	.049	**.311**	-.108
LL	-.071	.033	**.268**	.107
LW	.168	.089	-.065	-.176
Eigenvalues	4.97	4.42	2.88	2.06
% of Variance	29.21	26.01	16.97	12.11

[1/] GY = green yield, MDY = maketable dehusk yield, TDY = total dehusk yield, EL = ear length, SEL = seed ear length, EW = ear width, NSR = number of seed rows per ear, PH = plant height, EH = ear height, SPR1 = leaf greenness in silking stage, SPR3 = leaf greenness at milky stage, TAD = tassel date, SID = silking date, STD = stem diameter, LNO = leaf number, LL = leaf length, and LW = leaf width.

Grouping based on ear size (PC1), yield (PC2) and plant size (PC3) fixed total variation of 72.19% indicated that KKU1116 and KKU2901, bred varieties from Khon Kaen, showed quite similar characteristics whereas WPP was obviously separated from the other varieties due to different ear (PC1) from others. Both TSW and KWSX gave low yields and differed in plant type. Yield of GR484 variety was lower than that of PEK but equal to ear size of NSW (Figure 2). Principal component analysis was utilized to group desire characteristics accurately. This was useful for further breeding improvement for farmers and other stakeholders in production and usage (Okporie, 2008).

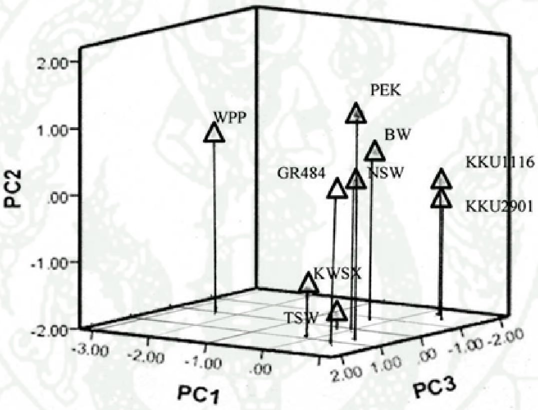

Figure 2 The distribution pattern of 9 waxy corn hybrids on 3 principal components, PC1, PC2 and PC3.

2.2 Cluster analysis

Genetic differences of waxy corn were calculated from 17 characteristics by Squared Euclidean Distance (Table 8). KKU1116 and KKU2901 were similarly characteristics with the least distance value of 5.55 whereas distance value of KWSX and TSW was 13.32. Distance value of WPP and the others was higher than the average value resulted in being isolated from the other groups. UPGMA dendogram grouping with distance value of 20.0 could separate the hybrids to be 3 groups (Figure 3).

Table 8 Squared euclidean distance among 9 waxy corn hybrid varieties based on 17 traits

Variety	**BW**	**WPP**	**PEK**	**GR**	**KWSX**	**KKU1116**	**KKU2901**	**TSW**	**Average**
NSW	26.81	50.56	41.71	30.44	25.26	38.88	36.11	18.47	33.53
BW		47.32	38.41	26.44	27.42	36.72	28.83	35.29	33.41
WPP			54.58	58.92	41.67	39.29	44.00	46.74	47.89
PEK				16.11	33.58	32.40	22.50	57.72	37.13
GR					18.73	36.62	27.79	41.67	32.09
KWSX						30.96	23.83	13.32	26.85
KKU1116							5.55	38.32	32.34
KKU2901								31.04	27.46

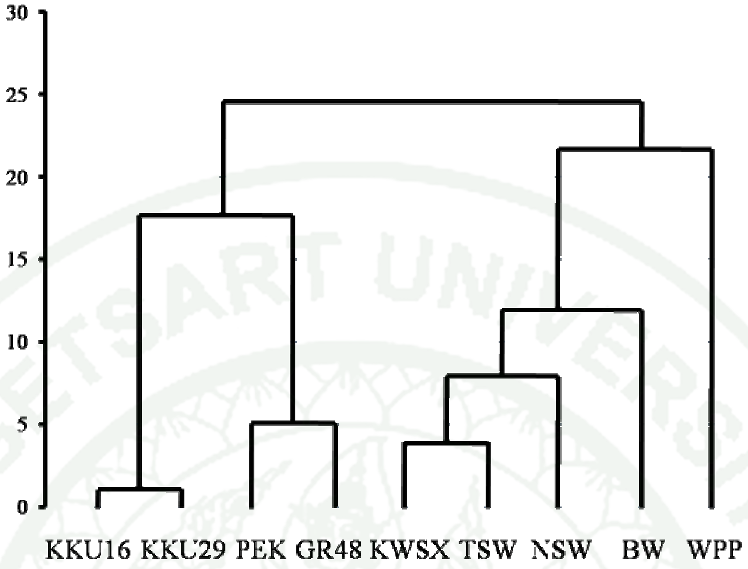

Figure 3 UPGMA dendogram for 9 hybrid parents based on the Euclidean distance matrix

Principal component scores were used for clustering samples into subgroups and discriminant analysis because a few principal components contained all the information in the original traits. The distribution pattern of 9 hybrids on PC1, PC2 and PC3 (Figure 2) was in agreement with the clustering pattern derived from the UPGMA dendogram (Figure 3) which was classified into 3 clusters. Cluster I comprised KKU1116, KKU2901, PEK and GR484 while cluster II comprised KWSX91, TSW, NSW and BW. Cluster III had only the WPP hybrid with different morphology from the others. Grouping by cluster method, total variables were used to obviously classify with overall consideration, but PC grouping could separate to consider only group having interesting characteristics and showing relationship such as ear size (PC1), yield (PC2) and plant size (PC3), or both ear size and plant size at the same time (Figure 2). Grouping based on quantitative traits had many advantages, because it quantified characters of potential parents and provided useful information for choosing parents in a breeding program (Van Beuningen and Busch, 1997)

2.3 Varietal cluster based on DNA

Twenty SSR primer pairs analyzed, 19 of them were polymorphic among the studied hybrids. Primer p-bnlg1092 was not polymorphic. A number of 144 alleles were detected from 9 hybrid varieties with 19 pairs of SSR primer. Four to 13 alleles of each primer pair were detected with an average of 7.6. The average PIC of 19 pairs of primer was 0.38 (range from 0.22 to 0.72) (Table 9) .The results were in notable of Cheng-lai *et al*. (2010) who revealed that the number of molecular markers of 6-304, materials of 12-2758 and polymorphic alleles of 110-802 were previously reported.

Genetic similarity (GS) revealed by Jaccard coefficients among the 9 hybrid varieties was calculated based on 144 polymorphic SSR alleles (Table 10). It was ranged from 0.261 to 0.684. UPGMA dendrograms based on Jaccard similarity, closely relationships were observed between BW and WPP (0.684), GR484 and KWSX (0.591) and KKU1116 and TSW (0.545). Nine hybrid varieties were roughly classified into four groups according to the similarity coefficient of 0.435 as a standard (Figure 4). The group-1 contained BW, WPP and KKU2901 while group II comprised GR484, KWSX91 and PEK and group III comprised TSW and KKU1116. The last group had only the NSW hybrid with genetic difference from the others. Grouping pattern by DNA marker method was different from that grouping by agronomic traits. The cluster results based on geographical distribution, phylogenetic relationships and molecular markers were not necessarily related to each other (Thomas *et al*., 1994). There were many reasons such as agronomic trait changing from environmental effects (Smith and Smith, 1989), number of SSR primer pairs and number of material (Chun-hong *et al*., 2010) affecting clustering pattern.

Table 9 The polymorphism information content of the 20 pairs of primers in 9 waxy corn hybrid varieties.

SSR primer	**Repeat**	**Bin no.**	**Allele no.**	**PIC**
bnlg1007.	AG(15)	1.02	10	0.44
umc1725		1.11	12	0.33
p-bnlg1092	(AG)30	2.01	0	-
p-umc1126	ACC	2.08	9	0.50
p-umc1394	(AT)10	3.01	9	0.50
p-bnlg1754	AG(20)	3.09	11	0.39
p-bnlg1370	AG(37)	4	5	0.72
p-bnlg1337	AG(21)	4.11	4	0.78
p-bnlg565		5.02	6	0.67
p-umc1225	(AG)6	5.08	5	0.72
p-bnlg249		6.01	6	0.67
p-umc1653	(GAAA)24	6.07	12	0.73
p-umc1426	(AGAGG)4	7	5	0.72
p-umc1671	(AGC)7	7.05	5	0.72
umc1359		8	5	0.72
p-umc1069	(GGAGA)6	8.08	6	0.67
p-phi033	AAG	9.01	13	0.28
p-umc1137	(CT)15	9.08	10	0.44
p-phi041	AGCC	10	6	0.67
p-bnlg1450	AG(34)	10.07	5	0.72
Average			7.2	0.60

Table 10 Jaccard similarity coefficients based on 19 SSR markers.

Variety	**BW**	**WPP**	**PEK**	**GR484**	**KWSX91**	**KKU1116**	**KKU2901**	**TSW**	**Average**
NSW	0.261	0.261	0.273	0.348	0.304	0.304	0.217	0.429	0.300
BW		0.684	0.409	0.360	0.320	0.375	0.409	0.320	0.392
WPP			0.409	0.478	0.375	0.500	0.476	0.375	0.445
PEK				0.435	0.455	0.455	0.304	0.280	0.378
GR484					0.591	0.400	0.435	0.296	0.418
KWSX91						0.478	0.280	0.308	0.389
KKU1116							0.333	0.545	0.424
KKU2901								0.333	0.348
TSW									0.361

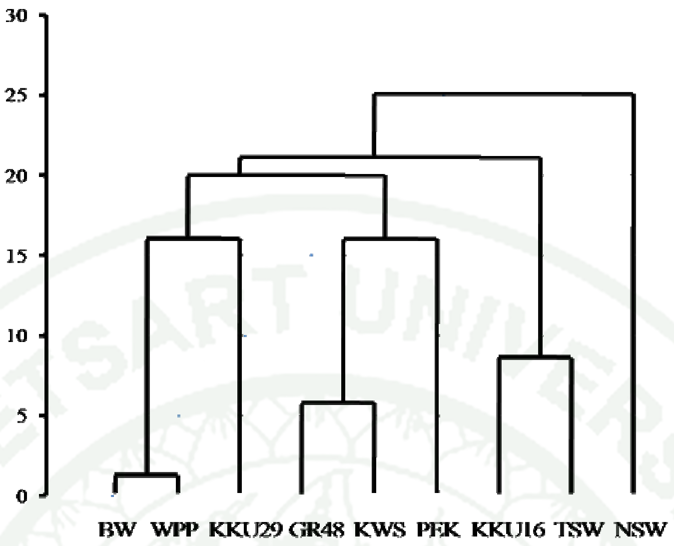

Figure 4 UPGMA dendogram for 9 hybrid parents based on 19 SSRs genetic similarity matrix measured by Jaccard method.

Cheng-lai *et al*. (2010) demonstrated that effects of the primer numbers on clustering results were mediated by the number of alleles. Therefore, in order to improve the reliability of cluster analysis, it is more important to increase the number of alleles than that of primers in genetic relationship study. Materials with closely genetic relationship were not clustered together. This might be caused by cross recombination and mutation during the selection (Chun-hong *et al*., 2010).

3. Genetic effect for yield based on analyses II and III

3.1 Combined analysis of variance for genetic effect

The combined analysis of variance over four environments based on analyses II and III (Gardner and Eberhart, 1966) (Table 11), indicated that all diallel effects significantly influenced yield.

For green yield (GY), variety effect (v_i) it was significant (p<0.01) but heterosis (h_{ij}) was not significant at the 5% level (p>0.05). For marketable dehusk yield (MDY), v_i and h_{ij} were significant (p<0.01), 40.9% and 59.1% of the total sum of squares due to differences among generation means could be explained by v_i and h_{ij}, respectively. These data suggested that additive and non additive effects were involved in the expression of yield in waxy corn. These results were different from results in grain yield of field corn reported by Crossa *et al*. (1987) who studied to identify superior exotic and exotic × adapted populations and indicated that v_i and h_{ij} were accounted for 60 and 40% of the total variation among population respectively for grain yield.

The heterosis parameter (h_{ij}) was the results of dominance gene action and difference in gene frequencies between parental varieties (Murray *et al*., 2003). The non-orthogonal partitioning revealed that average heterosis ($\hbar$), variety heterosis (h_i) and specific heterosis (s_{ij}) or specific combining ability (SCA) among hybrids were significant in MDY (Table 11). Even though SCA of MDY of the h_{ij} accounted for 28.3% of the total sum of squares was the most important and significant at the 5% level. Variety effects (v_i) and general combining ability (GCA) effects (g_i) were significantly different and larger than s_{ij} in GY and MDY which were corresponded to the work of Dickert and Tracy (2002) who studied in open pollinated sweet corn cultivars. The sum square of g_i and s_{ij} for MDY was 36.8% and 28.3 % of the total sum of squares, respectively.

Population × environment interactions were detected. Environments × v_i interactions, Environments × h_{ij} interactions and environments × g_i interactions were significant for both GY and MDY (Table 11).

Table 11 Mean square from combined analysis of variance for genetic effect tested in 4 environments.

SOV	df		GY[1]		MDY	
			% Among pop SS.	MS	% Among pop SS.	MS
Hybrids and Crosses (HC)	44		100.0	8.25 **[2]	100.0	6.84 **
Varieties (v_i)	8		51.7	23.44 **	40.9	15.35 **
Heterosis (h_{ij})	36		48.3	4.87 ^{ns}	59.1	4.94 **
Average ($\hbar$)		1	2.9	10.54 ^{ns}	14.5	43.54 **
Variety (h_i)		8	11.2	5.06 ^{ns}	16.3	6.13 *
Specific (s_{ij})		27	34.2	4.60 ^{ns}	28.3	3.16 **
GCA (g_i)	8		39.4	17.89 **	36.8	13.86 **
Env x HC	132			3.49 **		1.64 **
Env x v_i	24			4.80 **		2.72 **
Env x h_{ij}	108			3.20 **		1.40 **
Env x $\hbar$		3		7.42 **		0.58 ^{ns}
Env x h_i		24		2.37 ^{ns}		2.00 **
Env x s_{ij}		81		3.29 **		1.25 *
Env x g_i	24			3.68 **		2.27 **
Pooled Error		212		1.56		0.90
Total	431					
CV(%)				10.91		14.09

[1] GY = green yield, MDY = maketable dehusk yield

[2] ^{ns} = not significant differentce, * and ** = Significant at 0.05 and 0.01 probability level, respectively.

3.2 Genetic parameter effects across four environments

GY and MDY average over all varieties were 12.52 and 8.05 t/ha, respectively (Table 12). PEK, GR484, WPP, BW and KKU1116 showed positive varietal effects, *i.e.* these hybrids gave higher GY and MDY over the average of all varieties (Table 12). GCA effects (g_i) of PEK and NSW showed the highest GY and MDY, whereas that of TSW was the lowest. It could be explained that PEK and NSW were good combiners for GY and MDY. Heterotic effect of a variety in crosses can be measured as a deviation from average heterosis (Murray *et al*., 2003). KWSX91 showed the highest variety heterosis effect for GY (0.85 t/ha) and MDY (1.01 t/ha) (Table 12) but per se performance obtained the lowest of 6.43 t/ha for MDY (Table 12). NSW and BW showed high GCA effect and positive heterotic effect, however lower than KWSX91 in terms of heterotic effect. The positive variety heterosis of KWSX91, NSW and BW indicated differences in frequencies of dominant alleles between them and the other populations (Crossa *et al*., 1987). The correlation between GCA and variety effect was medium and low for GY (r = 0.62**) and MDY (r = 0.38*), respectively (Table 12). Thus, both variety effects and GCA effects should be considered to select for high yield germplasm. Desirable GCA effects and per se performance were observed in breeding materials used for germplasm improvement (Melchinger and Gumber, 1988). The correlation between variety heterosis and variety effect was negative in GY (r = -0.45**) and MDY (r = -0.54**) (Table 12).

Table 12 Variety effects (v_i), GCA effects (g_i) and variety heterosis (h_i) of parental waxy corn hybrid parents and correlation between variety and GCA effects ($r_{(vi,gi)}$) and variety heterosis ($r_{(vi,hi)}$) in yield.

Variety	**GY[1/] (t/ha)**			**MDY(t/ha)**		
	v_i	g_i	h_i	v_i	g_i	h_i
NSW	-0.39	0.58	0.77	-0.12	0.60	0.66
BW	0.87	0.12	-0.32	0.35	0.31	0.13
WPP	-0.04	0.06	0.08	0.58	0.12	-0.16
PEK	1.99	0.82	-0.17	1.44	0.65	-0.07
GR484	0.27	-0.46	-0.59	0.69	-0.35	-0.69
KWSX91	-1.06	0.32	0.85	-1.62	0.20	1.01
KKU1116	-0.08	-0.24	-0.20	0.19	-0.53	-0.63
KKU2901	0.51	-0.13	-0.38	-0.11	-0.22	-0.16
TSW	-2.07	-1.07	-0.04	-1.39	-0.77	-0.08
Average	12.52	12.09	-0.43	8.05	7.18	-0.87
F-test	** [2/]	**	ns	**	**	*
S.E. [3/]	1.25	0.36	1.01	0.95	0.27	0.77
$r_{(vi,gi)}$		0.62**			0.38*	
$r_{(vi,,hi)}$		-0.45**			-0.54**	

[1/] GY = green yield, MDY = maketable dehusk yield,

[2/] ns = non significant, * and ** = significant at 0.05 and 0.01 probability level, respectively.

[3/] S.E. = standard error of the mean effects.

Environments × v_i interactions were significant for GY and MDY. PEK showed the highest estimate of variety effect in the combined data in all environments consistently. BW had high estimate of variety effect in ENV1 and ENV 4 but low in ENV2 (Table 13). WPP showed high estimate of variety effect in ENV2, ENV3 and ENV4 but low estimate in ENV1. KWSX19 showed negative variety effect in all environments. The interaction effect of ENV was due to sequence changes in each different environment such as differences in fertilizer application (Medici *et al*., 2004).

Table 13 Variety effects (v_i) from Gardner and Eberhart model analysis III and their ranks for MDY (t/ha) in 4 environments and combined over 4 environments.

Variety	**ENV1**[1/]		**ENV2**		**ENV3**		**ENV4**		**Combined ENV.**	
	v_i	**Rank**	v_i	**Rank**	v_i	**Rank**	v_i	**Rank**	v_i	**Rank**
NSW	0.87	3	-0.14	6	-0.72	7	-0.49	7	-0.12	7
BW	2.62	1	-1.94	8	-0.07	5	0.81	2	0.35	4
WPP	-1.13	7	1.06	3	1.73	2	0.66	3	0.58	3
PEK	1.07	2	2.41	1	1.93	1	0.36	4	1.44	1
GR484	0.12	4	1.41	2	-0.62	6	1.86	1	0.69	2
KWSX19	-1.28	9	-2.19	9	-1.72	8	-1.29	8	-1.62	9
KKI1116	-1.18	8	0.91	4	1.28	3	-0.24	6	0.19	5
KKU2901	-0.68	6	-0.04	5	0.18	4	0.11	5	-0.11	6
TSW	-0.38	5	-1.44	7	-1.97	9	-1.74	9	-1.39	8
μ_v	8.48		7.14		8.47		8.09		8.05	

[1/] Env1 = Suwan farm in rainy season (Aug-Oct.2008), Env2 = Suwan farm in dry season (Dec.-Feb.2009), Env3 = RMUTSB in dry season (Dec.-Feb.2009), and Env4 = RMUTSB in early rainy season (May-June.2009)

Environments × g_i interactions for GY and MDY in this study were also different. Ranking of GCA effects for MDY varied among environments (Table 14). NSW showed positive GCA effect in all environments. The highest estimate was ranked in ENV2 and ENV3, however it was ranked as the second in the combined data. While estimate of GCA of PEK showed the first rank in the combined data but low estimate in ENV3 whereas TSW had low estimate consistently.

Environments × h_i interactions were significant for MDY. KWSX91 gave the highest estimate of variety heterosis effect in the combined data in all environments, except ENV4. NSW also showed high estimate of variety heterosis effect in all environments (Table 15). PEK showed the highest estimate of variety heterosis effect in ENV1 but the lowest in ENV3. GR484 and KKU1116 showed negative variety heterosis effect in all environments. The average heterosis ($\hbar$) for MDY showed negative effect in all environments.

The average heterosis ($\hbar$) for MDY was negative (-0.87) (Table 15), MDY averaged over 36 double cross combinations (DC) was 7.18 ton/ha which was less than that of 8.05 t/ha of the hybrid parents due to inbreeding depression in the DC (Table 16). A similar result was observed in grain yield of field corn (Phumichai *et al.*, 2008).

The specific heterosis (s_{ij}) or SCA for MDY ranged from -1.14 to 1.08 t/ha (Table 16). BW/PEK showed the highest yielding of DC (9.17 t/ha) with the s_{ij} of 1.04. This DC parents exhibited high g_i estimate for MDY. The MDY of NSW/BW, NSW/PEK, NSW/KKU2901 and PEK/WPP gave high yield over 8 t/ha and their s_{ij} exhibited positive significance were 0.69, 0.33 and 0.55 and 0.40 t/ha, respectively (Table 16). Heterosis in DC was probably due to the difference between genotypes of the parental single crosses. The expression of heterosis depended on the difference in allele frequency of the parents and dominant effect at various loci (Falconer and Mackay, 1996).

Environments × s_{ij} interactions were significant for GY and MDY. The data of GY and MDY were similarly. Therefore, the result of Environments × s_{ij} interactions was shown only MDY. In the absence of epistasis, the Gardner Eberhart model was used to explain heterosis in terms of dominance and the square of the difference in gene frequency between parents. Such a parameter provided an estimate of gene variability which was an important consideration in choosing germplasm pools to be used in recurrent selection programs (Crossa *et al*., 1987). The specific heterosis effect (s_{ij}) was detected when genetically distinct hybrids were used to generate DC (Moll *et al*., 1965; Troyer *et al*., 1988). Six DCs namely KWSX19/KKU1116, BW/PEK, KWSX19/ KKU2901, NSW/ KKU2901, WPP/ PEK and NSW/ TSW exhibited positive estimate of s_{ij} for MDY in all environments (Table 16). KWSX19/KKU1116 showed the highest s_{ij} and that of BW/PEK was the second rank. They all exhibited high estimate of s_{ij} in the combined data and showed a positive effect estimate in all environments. Estimation of s_{ij} of GR484/TSW showed the third rank in the combined data but not consistently. GR484/TSW showed positive effects and high values in ENV 2, ENV 3 and ENV 4, but low estimates in ENV1. KKU1116/ KKU2901 and WPP/TSW showed negative s_{ij} in all environments and had low estimates consistently (Table 17). Such a poor performance was expected, since the parents were closely related to origin (Dhillon and Singh, 1977).

Table 14 GCA effects (g_i) from Gardner and Eberhart model analysis II and their ranks for MDY (ton/ha) in 4 environments and combined over 4 environments

Variety	**ENV1**[1/]		**ENV2**		**ENV3**		**ENV4**		**Combined ENV**	
	g_i	**Rank**	g_i	**Rank**	g_i	**Rank**	g_i	**Rank**	g_i	**Rank**
NSW	0.57	2	0.79	1	0.82	1	0.21	3	0.58	2
BW	0.17	3	0.08	5	-0.06	6	1.05	2	0.31	3
WPP	-0.05	5	-0.07	6	0.70	2	-0.09	6	0.12	5
PEK	0.87	1	0.47	2	-0.03	5	1.27	1	0.65	1
GR484	-0.44	8	0.19	4	-0.40	8	-0.74	8	-0.35	7
KWSX19	0.10	4	0.30	3	0.46	3	-0.08	5	0.20	4
KKI1116	-0.68	9	-0.51	7	-0.22	7	-0.73	7	-0.53	8
KKU2901	-0.33	7	-0.52	8	-0.01	4	-0.01	4	-0.22	6
TSW	-0.23	6	-0.72	9	-1.27	9	-0.87	9	-0.77	9
μ_c	7.82		6.05		7.72		7.13		7.18	
S.E. g_i	0.28		0.31		0.41		0.43		0.36	

[1/] Env1 = Suwan farm in rainy season (Aug-Oct.2008), Env2 = Suwan farm in dry season (Dec.-Feb.2009), Env3 = RMUTSB in dry season (Dec.-Feb.2009), Env4 = RMUTSB in early rainy season (May-June.2009)

Table 15 Variety heterosis effects (h_i) from Gardner and Eberhart model analysis II and their ranks for MDY (t/ha) in 4 environments and combined over 4 environments.

Variety	**ENV1**[1/]		**ENV2**		**ENV3**		**ENV4**		**Combined ENV**	
	h_i	**Rank**	h_i	**Rank**	h_i	**Rank**	h_i	**Rank**	h_i	**Rank**
NSW	0.14	4	0.86	3	1.19	2	0.45	4	0.66	2
BW	-1.13	9	1.05	2	-0.03	3	0.65	2	0.13	3
WPP	0.52	2	-0.59	7	-0.16	6	-0.42	7	-0.16	7
PEK	0.34	3	-0.73	8	-0.99	9	1.09	1	-0.07	4
GR484	-0.50	8	-0.51	6	-0.09	4	-1.67	9	-0.69	9
KWSX91	0.74	1	1.39	1	1.32	1	0.57	3	1.01	1
KKU1116	-0.08	7	-0.96	9	-0.86	8	-0.61	8	-0.63	8
KKU2901	0.01	5	-0.50	5	-0.10	5	-0.06	6	-0.16	6
TSW	-0.04	6	0.00	4	-0.28	7	0.00	5	-0.08	5
Average heterosis ($\hbar$)	-0.66		-1.10		-0.75		-0.97		-0.87	

[1/] Env1 = Suwan farm in rainy season (Aug-Oct.2008), Env2 = Suwan farm in dry season (Dec.-Feb.2009), Env3 = RMUTSB in dry season (Dec.-Feb.2009), Env4 = RMUTSB in early rainy season (May-June.2009)

Table 16 Marketable dehusk yield (t/ha) of 36 double crosses (below diagonal), 6 parental hybrids (on diagonal) and specific heterosis effects (s_{ij}) of double crosses (above diagonal)

Variety	**NSW**	**BW**	**WPP**	**PEK**	**GR484**	**KWSX91**	**KKU1116**	**KKU2901**	**TSW**
NSW	**7.93**	0.69	-0.13	-0.33	-0.66	-0.47	0.03	0.55	0.31
BW	8.76	**8.40**	0.25	1.04	-0.58	-0.62	0.41	-0.62	-0.56
WPP	7.78	7.88	**8.63**	0.40	0.28	0.14	-0.18	0.38	-1.14
PEK	8.10	9.17	8.35	**9.49**	0.11	-0.32	-0.47	-0.63	0.20
GR484	6.77	6.57	7.23	7.58	**8.74**	-0.50	0.08	0.46	0.81
KWSX91	7.51	7.06	7.64	7.69	6.53	**6.43**	1.08	0.62	0.08
KKU1116	7.27	7.34	6.59	6.84	6.38	7.92	**8.24**	-1.01	0.07
KKU2901	8.12	6.64	7.47	6.98	7.08	7.77	5.41	**7.94**	0.25
TSW	7.31	6.15	5.39	7.25	6.85	6.67	5.92	6.43	**6.66**

S.E.s_{ij} = 0.78 t/ha, Double cross combinations mean averaged over 36 DC = 7.18 t/ha,

Parental mean averaged over 9 parents = 8.05 t/ha.

LSD $_{0.05}$ and LSD$_{0.01}$ for dehusk yield of double cross combinations and parent hybrids are 1.25 and 1.61 t/ha, respectively.

Table 17 Specific heterosis effects (s_{ij}) from Gardner and Eberhart model analysis II and their ranks for MDY (t/ha) in 4 environments and combined over 4 environments.

Double cross	ENV1	Rank	ENV2	Rank	ENV3	Rank	ENV4	Rank	Combined ENV	Rank
NSW/BW	0.18	17	1.39	4	-0.29	25	1.47	3	0.69	4
NSW/WPP	-0.35	27	0.08	15	0.70	7	-0.94	29	-0.13	22
NSW/ PEK	0.18	18	-0.81	30	-0.77	30	0.10	18	-0.33	25
NSW/ GR484	-0.81	31	-0.53	28	-0.84	31	-0.44	25	-0.66	34
NSW/ KWSX19	-0.65	30	-0.83	31	-0.06	22	-0.35	23	-0.47	27
NSW/ KKU1116	0.43	10	-0.43	25	1.02	4	-0.90	27	0.03	21
NSW/ KKU2901	0.44	9	0.89	5	0.06	18	0.83	8	0.55	6
NSW/ TSW	0.59	5	0.24	11	0.17	14	0.24	17	0.31	11
BW/WPP	0.25	13	-0.01	16	0.34	11	0.42	15	0.25	14
BW/PEK	0.53	7	2.01	1	0.76	6	0.86	7	1.04	2
BW/ GR484	0.19	16	-0.36	22	-0.26	24	-1.89	36	-0.58	30
BW/ KWSX19	0.10	20	-0.47	26	-0.42	27	-1.69	34	-0.62	32
BW/ KKU1116	-0.12	22	-0.36	23	-0.44	28	2.56	1	0.41	8
BW/ KKU2901	-0.86	34	-1.05	34	0.20	12	-0.77	26	-0.62	31
BW/ TSW	-0.26	25	-1.15	36	0.11	17	-0.95	30	-0.56	29
WPP/ PEK	0.15	19	0.20	12	0.65	9	0.60	11	0.40	9
WPP/ GR484	0.56	6	0.78	6	0.13	16	-0.34	22	0.28	12
WPP/ KWSX19	-0.38	28	-0.28	21	0.06	19	1.15	5	0.14	16

Table 17 (Continued)

Double cross	**ENV1**	**Rank**	**ENV2**	**Rank**	**ENV3**	**Rank**	**ENV4**	**Rank**	**Combined ENV**	**Rank**
WPP/ KKU1116	0.30	12	-0.37	24	-1.26	35	0.60	10	-0.18	23
WPP/ KKU2901	-0.34	26	0.64	8	1.24	2	-0.02	19	0.38	10
WPP/ TSW	-0.19	24	-1.06	35	-1.86	36	-1.46	33	-1.14	36
PEK/ GR484	-1.06	36	-0.51	27	0.16	15	1.84	2	0.11	17
PEK/ KWSX19	-0.45	29	-0.06	17	-0.56	29	-0.22	20	-0.32	24
PEK/ KKU1116	-0.17	23	0.14	14	-0.13	23	-1.72	35	-0.47	26
PEK/ KKU2901	-0.06	21	-0.24	20	-1.19	34	-1.04	31	-0.63	33
PEK/ TSW	0.89	2	-0.74	29	1.07	3	-0.42	24	0.20	15
GR484/ KWSX19	0.21	14	-0.98	33	-0.33	26	-0.91	28	-0.50	28
GR484/ KKU1116	0.74	3	-0.13	18	-0.05	21	-0.26	21	0.08	18
GR484/ KKU2901	1.00	1	-0.16	19	0.19	13	0.82	9	0.46	7
GR484/ TSW	-0.85	33	1.89	2	1.00	5	1.18	4	0.81	3
KWSX19/ KKU1116	0.60	4	1.67	3	1.54	1	0.53	13	1.08	1
KWSX19/ KKU2901	0.21	15	0.68	7	0.68	8	0.91	6	0.62	5
KWSX19/ TSW	0.36	11	0.28	10	-0.91	32	0.58	12	0.07	19
KKU1116/ KKU2901	-0.81	32	-0.91	32	-1.14	33	-1.19	32	-1.01	35
KKU1116/ TSW	-0.96	35	0.39	9	0.46	10	0.38	16	0.07	20
KKU2901/ TSW	0.44	8	0.15	13	-0.04	20	0.46	14	0.25	13
S.E. s_{ij}	0.67		0.75		1.00		1.04		0.78	

4. Genetic effect for Ear size base on analyses II and III

4.1 Combined analysis of variance for genetic effect

Variety effects (v_i) and heterosis effect (h_{ij}) of ear length accounted for 80% and 20% of the total sum of squares, respectively and were highly significant ($p<0.01$) (Table 18). The h_{ij} was divided to be average heterosis ($\hbar$), variety heterosis (h_i) and specific heterosis (s_{ij}), they were 0.4, 9.7 and 10.1% of the total sum of squares, respectively. The h_{ij} and s_{ij} were highly significant ($p<0.01$) but $\hbar$ was not significant ($p>0.05$). For ear width, v_i and h_{ij} accounted for 90% and 10% of the total sum of squares, respectively and were highly significant ($p<0.01$). Only h_i was highly significant ($p<0.01$) and accounted for 4.4 % of the total sum of squares. The GCA effect (g_i) was significantly different for EL, EW and SRN. The number of seed row of v_i and g_i, except h_{ij} significantly exhibited. The data suggested that additive and non additive effects were involved in the expression of ear size in waxy corn, however additive effects were more important than non additive effects. The significance of GCA effects indicated that the least one of lines was different in content of favorable genes with additive effects. While the differences of SCA indicated complementation between lines at loci with some degrees of non additive effect (Medici *et al*., 2004).

Populations × environment interactions were detected for EL, EW and SRN. Environments × v_i and Environments × g_i were significant ($p<0.05$) but environments × h_{ij} was not significant ($p>0.05$).

Table 18 Mean square from combined analysis of variance for genetic effect tested in 4 environments.

SOV	Df	EL[1/]			EW			SRN		
		% Among pop SS.	MS		% Among pop SS.	MS		% Among pop SS.	MS	
Hybrids and Crosses (HC)	44	100.0	7.61	**[2/]	100.0	0.70	**	100.0	6.14	**
Varieties (v_i)	8	79.7	33.37	**	89.5	3.45	**	83.3	28.15	**
Heterosis (h_{ij})	36	20.2	1.88	**	10.5	0.09	**	16.7	1.25	ns
Average ($\hbar$)	1	0.4	1.46	ns	0.0	0.00	ns	0.1	0.18	ns
Variety (h_i)	8	9.7	4.06	**	4.4	0.17	**	6.0	2.02	**
Specific (s_{ij})	27	10.1	1.25	**	6.1	0.07	ns	10.6	1.06	*
GCA (g_j)	8		17.32	**		1.66	**		18.16	**
Env x HC	132		0.81	*		0.05	**		0.78	**
Env x v_i	24		1.72	**		0.10	**		1.60	**
Env x h_{ij}	108		0.61	ns		0.04	ns		0.59	ns
Env x $\hbar$	3		1.11	ns		0.03	ns		0.73	ns
Env x h_i	24		0.74	ns		0.04	ns		0.38	ns
Env x s_{ij}	81		0.55	ns		0.05	ns		0.65	ns
Env x g_i	24		1.30	**		0.07	*		1.29	**
Pooled Error	212		0.62			0.04			0.51	
Total	431									
CV (%)			4.86			4.69			5.36	

[1/] EL = ear length, EW = ear width, NSR = number of seed rows per ear

[2/] ^{ns} = not significant differentce (p>0.05), * and ** = Significant at 0.05 and 0.01 probability level, respectively.

4.2 Genetic parameter effects across four environments

Average ear length (EL), ear width (EW) and number of seed row (NSR) of hybrid varieties was 16.31, 4.19 cm and 13.47 rows, respectively (Table 19). WPP showed both the widest and shortest ear, the best general combiner for EW and NSR but the worst GCA effect (g_i) for EL. Large GCA effects of the parents were mainly due to additive and additive × additive gene actions (Griffing, 1956). The correlation between GCA and variety effect was high for EL (r = 0.83**) and EW (r = 0.97**) (Table 19). Thus, the parents could be chosen based on variety effect for ear size.

Variety heterosis (h_i) of parent was significantly different for EL and EW, except for NSR. WPP had high positive heterotic effect for EL but showed negative effect for EW and NSR. WPP was the worst general combiner but exhibited the highest variety heterosis effect (0.46 cm) for EL (Table 19). WPP was a good general combiner but the lowest EW of -0.18 cm (Table 19). TSW was a small ear variety and the worst general combiner for the both of EL and EW but had high h_i. The correlation between variety heterosis and variety effect was negative in all traits, particularly for EL (r = -0.73**) and EW (r = -0.88**) (Table 19).

For ear size, population × environment interaction was detected. Environments × v_i interactions and environments × g_i interactions were significantly different for all traits but environment × h_i interaction was not different (Table 18).

Table 19 Variety effects (v_i), GCA effects (g_i) and variety heterosis (h_i) of parental waxy corn hybrid parents and correlation between variety effects**,** GCA effects ($r_{(vi,gi)}$) and variety heterosis ($r_{(vi,hi)}$) in yield component traits.

Variety	**EL[1/] (cm)**			**EW (cm)**			**NSR(row)**		
	v_i	g_i	h_i	v_i	g_i	h_i	v_i	g_i	h_i
NSW	0.33	0.56	0.40	-0.19	-0.02	0.08	-0.27	0.12	0.25
BW	-0.37	-0.19	0.00	0.09	0.04	0.00	-0.82	-0.62	-0.20
WPP	-3.05	-1.07	0.46	1.18	0.41	-0.18	3.03	1.06	-0.45
PEK	1.56	0.83	0.04	-0.10	0.00	0.05	-0.57	-0.24	0.05
GR484	1.86	0.04	-0.90	-0.07	-0.13	-0.10	-0.57	-0.45	-0.16
KWSX91	0.20	0.23	0.13	0.06	0.00	-0.03	-0.17	-0.20	-0.11
KKU1116	1.09	0.13	-0.42	-0.05	-0.01	0.02	0.63	0.40	0.08
KKU2901	0.23	-0.05	-0.17	-0.25	-0.08	0.04	-0.97	-0.59	-0.11
TSW	-1.84	-0.47	0.45	-0.67	-0.21	0.12	-0.27	0.52	0.65
Average	16.31	16.47	0.16	4.19	4.20	0.01	13.47	13.42	-0.06
F-test	**[2/]	**	**	**	**	**	**	**	ns
S.E.	0.78	0.22	0.63	0.19	0.06	0.16	0.71	0.20	0.58
$r_{(vi,gi)}$		0.83			0.97			0.28	
$r_{(vi,hi)}$		-0.73			-0.88			-0.39	

[1/] EL = ear length, EW = ear width, NSR = number of seed rows per ear

[2/] ns = non significant, * and ** = significant at 0.05 and 0.01 probability level respectively.
SE. = standard error of the mean effects.

The variety effect (v_i) average across four environments of GR484, PEK and KKU1116 for EL were 1.86, 1.56 and 1.09 cm (Table 20), respectively and showed positive effects in all environments. The variety effect in the combined data of GR484 was the highest but g_i value of 0.04 cm was lower than some varieties (Table 21). PEK was the second rank v_i for EL and showed the highest g_i (0.83 cm) in the combined data and also had a consistently high estimate in all environments (Table 21). While EL of WPP, TSW and BW showed negative v_i and g_i in the combined data and had consistently low estimate in all environments. WPP showed the lowest v_i and g_i in all environments for EL. The v_i and g_i across four environments of WPP were -3.05 (Table 20) and -1.07 cm (Table 21) for EL, respectively.

Table 20 Variety effects (v_i) from Gardner and Eberhart model analysis III and their ranks for EL (cm) in 4 environments and combined across 4 environments.

Variety	ENV1[1/]		ENV2		ENV3		ENV4		Combined Env	
	v_i	Rank	v_i	Rank	v_i	Rank	v_i	Rank	v_i	Rank
NSW	1.68	3	0.64	5	0.12	5	-1.14	7	0.33	4
BW	-0.17	6	-0.91	7	-0.53	7	0.11	6	-0.38	7
WPP	-3.77	9	-2.56	9	-2.33	9	-3.54	9	-3.05	9
PEK	2.28	1	1.09	2	1.82	1	1.06	2	1.56	2
GR484	1.93	2	0.69	4	1.37	2	3.46	1	1.86	1
KWSX19	0.08	5	-0.06	6	0.27	4	0.51	4	0.2	6
KKU1116	0.93	4	1.44	1	1.27	3	0.71	3	1.09	3
KKU2901	-0.37	7	0.69	3	0.07	6	0.51	5	0.23	5
TSW	-2.57	8	-1.06	8	-2.08	8	-1.64	8	-1.84	8
μ_v	16.2		15.6		16.8		16.7		16.3	

Table 21 GCA effects (g_i) from Gardner and Eberhart model analysis II and their ranks for EL (cm) in 4 environments and combined across 4 environments.

Variety	ENV1[1/]		ENV2		ENV3		ENV4		Combined Env	
	g_i	Rank	g_i	Rank	g_i	Rank	g_i	Rank	g_i	Rank
NSW	0.70	2	0.69	1	0.78	1	0.08	5	0.56	2
BW	-0.35	6	-0.43	8	-0.44	7	0.48	3	-0.19	7
WPP	-1.10	9	-0.92	9	-1.04	9	-1.22	9	-1.07	9
PEK	1.14	1	0.62	2	0.58	2	0.96	1	0.83	1
GR484	-0.04	5	0.08	5	0.15	3	-0.05	6	0.04	5
KWSX19	0.22	4	0.24	3	-0.01	6	0.48	2	0.23	3
KKI1116	0.57	3	0.13	4	0.21	5	-0.40	7	0.13	4
KKU2901	-0.44	7	-0.20	6	0.26	4	0.15	4	-0.05	6
TSW	-0.70	8	-0.21	7	-0.50	8	-0.48	8	-0.47	8
μ_c	16.18		15.81		17.31		16.59		16.47	
S.E.g_i	0.27		0.26		0.27		0.30		0.30	

[1/] Env1 = Suwan farm in rainy season, Env2 = Suwan farm in dry season,
Env3 = RMUTSB in dry season, Env4 = RMUTSB in early rainy season

Environments × v_i interactions and environments × g_i interactions were significant for EW. WPP showed the highest estimate of v_i (1.18 cm) (Table 22) and g_i (0.41 cm) (Table 23) in the combined data and had a consistently high estimate in all environments. Interaction of environments with v_i was observed in BW, which had high estimate of v_i and g_i in ENV1 and ENV4 but low in ENV2 (Tables 22, 23). KWSX19 had high estimate of v_i in ENV2 and ENV3 but low in ENV1. TSW and KKU2901 showed negative v_i and g_i in all environments. TSW showed the lowest estimate of v_i and g_i in the combined data and had a consistently low estimate in all environments.

Table 22 Variety effects (v_i) from Gardner and Eberhart model analysis III and their ranks for EW (cm) in 4 environments and combined across 4 environments.

Variety	ENV1[1/]		ENV2		ENV3		ENV4		Combined Env	
	v_i	Rank	v_i	Rank	v_i	Rank	v_i	Rank	v_i	Rank
NSW	0.03	3	-0.13	6	-0.27	7	-0.38	8	-0.19	7
BW	0.38	2	-0.13	7	-0.12	5	0.22	2	0.09	2
WPP	1.13	1	1.07	1	1.43	1	1.07	1	1.18	1
PEK	-0.37	8	-0.08	5	-0.02	4	0.07	3	-0.10	6
GR484	-0.07	4	0.07	4	-0.12	6	-0.18	7	-0.07	5
KWSX19	-0.12	5	0.12	2	0.23	2	0.02	4	0.06	3
KKI1116	-0.22	6	0.07	3	-0.02	3	-0.03	5	-0.05	4
KKU2901	-0.37	7	-0.23	8	-0.32	8	-0.08	6	-0.25	8
TSW	-0.42	9	-0.78	9	-0.82	9	-0.68	9	-0.67	9
μ_v	3.87		4.33		4.37		4.18		4.19	

[1/] Env1 = Suwan farm in rainy season, Env2 = Suwan farm in dry season,
Env3 = RMUTSB in dry season, Env4 = RMUTSB in early rainy season

Table 23 GCA effects (g_i) from Gardner and Eberhart model analysis II and their ranks for EW (cm) in 4 environments and combined across 4 environments.

Variety	ENV1[1/]		ENV2		ENV3		ENV4		Combined Env	
	g_i	Rank	g_i	Rank	g_i	Rank	g_i	Rank	g_i	Rank
NSW	-0.04	4	0.05	3	-0.06	6	-0.02	6	-0.02	6
BW	0.10	2	-0.08	6	0.02	3	0.12	2	0.04	2
WPP	0.36	1	0.40	1	0.48	1	0.39	1	0.41	1
PEK	0.01	3	0.02	5	-0.11	7	0.06	3	0.00	3
GR484	-0.16	9	-0.09	7	-0.16	8	-0.12	7	-0.13	8
KWSX19	-0.10	8	0.08	2	0.00	5	0.03	4	0.00	4
KKI1116	-0.06	6	0.02	4	0.03	2	-0.01	5	-0.01	5
KKU2901	-0.05	5	-0.11	8	-0.02	4	-0.14	8	-0.08	7
TSW	-0.07	7	-0.28	9	-0.18	9	-0.32	9	-0.21	9
μ_c	3.85		4.28		4.41		4.24		4.20	
S.E.g_i	0.09		0.06		0.07		0.06		0.08	

[1/] Env1 = Suwan farm in rainy season, Env2 = Suwan farm in dry season, Env3 = RMUTSB in dry season, Env4 = RMUTSB in early rainy season

Environments × v_i interactions and environments × g_i interactions were significant for NSR. WPP had the highest estimate of v_i and g_i in all environments and had consistently high estimate. Variety effect averaged across four environments of WPP was 3.03 rows per ear (Table 24) while GCA effect was 1.06 rows per ear (Table 25). The second rank of v_i was found in KKU1116 showing positive estimate of v_i in all environments. BW and KKU2901 showed negative v_i in all environments. Interaction between Environments and v_i was observed in TSW, which had high estimate of v_i in ENV1 but low in ENV2 and ENV3 and medium in ENV4 (Table 24). TSW gave positive estimate of g_i in all environments and ranked as the second in the combined data. TSW showed low interaction of g_i for SRN with the highest value in ENV1, the second value in ENV3 and ENV4 and the third value in ENV2 (Table 25).

Table 24 Variety effects (v_i) from Gardner and Eberhart model analysis III and their ranks for NSR (row) in 4 environments and combined across 4 environments.

Variety	**ENV1[1/]**		**ENV2**		**ENV3**		**ENV4**		**Combined Env**	
	v_i	**Rank**	v_i	**Rank**	v_i	**Rank**	v_i	**Rank**	v_i	**Rank**
NSW	-0.42	5	0.16	3	-0.93	6	0.11	3	-0.27	4
BW	-0.82	7	-0.64	7	-1.53	9	-0.29	6	-0.82	8
WPP	2.58	1	2.76	1	4.27	1	2.51	1	3.03	1
PEK	-1.02	9	-0.84	9	0.27	3	-0.69	8	-0.57	6
GR484	-0.62	6	-0.44	4	-0.53	5	-0.69	7	-0.57	7
KWSX19	-0.02	4	-0.64	6	-0.13	4	0.11	4	-0.17	3
KKI1116	0.38	3	0.96	2	0.67	2	0.51	2	0.63	2
KKU2901	-0.82	8	-0.44	5	-0.93	7	-1.69	9	-0.97	9
TSW	0.78	2	-0.84	8	-1.13	8	0.11	5	-0.27	5
μ_v	14.02		13.24		13.13		13.49		13.47	

Table 25 GCA effects (g_i) from Gardner and Eberhart model analysis II and their ranks for NSR (row) in 4 environments and combined across 4 environments.

Variety	**ENV1[1/]**		**ENV2**		**ENV3**		**ENV4**		**Combined Env**	
	g_i	**Rank**	g_i	**Rank**	g_i	**Rank**	g_i	**Rank**	g_i	**Rank**
NSW	-0.01	5	0.40	4	-0.23	4	0.30	3	0.12	4
BW	-0.52	9	-0.77	9	-0.76	9	-0.41	7	-0.62	9
WPP	0.51	2	0.83	1	1.87	1	1.05	1	1.06	1
PEK	-0.32	7	-0.08	5	-0.42	5	-0.12	6	-0.24	6
GR484	-0.35	8	-0.51	8	-0.46	6	-0.47	8	-0.45	7
KWSX19	0.22	3	-0.48	7	-0.46	7	-0.07	5	-0.20	5
KKI1116	0.22	4	0.57	2	0.48	3	0.30	4	0.40	3
KKU2901	-0.29	6	-0.43	6	-0.62	8	-1.04	9	-0.59	8
TSW	0.54	1	0.46	3	0.62	2	0.45	2	0.52	2
μ_c	13.71		13.12		13.36		13.48		13.42	
S.E.g_i	0.28		0.24		0.26		0.19		0.28	

[1/] Env1 = Suwan farm in rainy season, Env2 = Suwan farm in dry season,
Env3 = RMUTSB in dry season, Env4 = RMUTSB in early rainy season

Ear size did not exhibit environments × specific heterosis (s_{ij}) interactions. These effects were calculated as the average across all environments. WPP was the shortest and widest ear of 13.26 and 5.36 cm, respectively, and showed the highest value of SRN. WPP had the highest estimate effect of variety heterosis (0.46 cm) for EL (Table 26) but the lowest for EW (-0.18 cm) (Table 27) and SRN (-0.45 cm) (Table 28). WPP was the parent of BW/WPP which exhibited the highest effect of s_{ij} for EL (Table 26) while WPP/GR484 exhibited the lowest effect of s_{ij} for EW (Table 27). NSW/PEK showed the longest ear of 18.04 cm and was also superior to its parents. NSW also had high estimate effect of 0.40 cm of variety heterosis while that of PEK was in the middle of 0.04 cm for EL. WPP and TSW were parents of short ear variety. WPP/TSW showed the shortest ear DC of 14.18 cm and had low specific heterosis effects (-0.76).

Table 26 Ear length (cm) of 36 double crosses (below diagonal), 6 parent hybrids (on diagonal), specific heterosis effects (s_{ij}) of double crosses (above diagonal) and variety heterosis (h_i).

Variety	**NSW**	**BW**	**WPP**	**PEK**	**GR484**	**KWSX91**	**KKU1116**	**KKU2901**	**TSW**
NSW	**16.64**	-0.06	0.13	0.18	-0.46	0.05	-0.15	0.03	0.28
BW	16.79	**15.94**	0.68	-0.08	-0.55	-0.09	0.48	-0.19	-0.19
WPP	16.10	15.90	**13.26**	0.08	0.49	0.40	-0.83	-0.21	-0.76
PEK	18.04	17.03	16.31	**17.88**	0.07	-0.24	-0.12	0.01	0.11
GR484	16.61	15.78	15.93	17.40	**18.18**	-0.56	0.05	0.42	0.54
KWSX91	17.31	16.43	16.04	17.29	16.18	**16.51**	0.35	0.08	0.02
KKU1116	17.01	16.89	14.70	17.30	16.69	17.18	**17.40**	0.05	0.17
KKU2901	17.01	16.04	15.14	17.25	16.88	16.73	16.59	**16.54**	-0.18
TSW	16.85	15.63	14.18	16.94	16.58	16.25	16.30	15.76	**14.48**
h_i	0.40	0.00	0.46	0.04	-0.90	0.13	-0.42	-0.17	0.45

Double cross combination mean = 16.47 cm, Parental mean = 16.31 cm, Average heterosis = 0.16 cm.

$LSD_{0.05}$ and $LSD_{0.01}$ for ear length of double cross combinations and parental hybrids are 0.90 and 1.19 cm, respectively.

S.E.s_{ij} = 0.72,   S.E.h_i = 0.63

Table 27 Ear width (cm) of 36 double crosses (below diagonal) 9 parental hybrids (on diagonal) specific heterosis effects (s_{ij}) of double crosses (above diagonal) and variety heterosis (h_i).

Variety	**NSW**	**BW**	**WPP**	**PEK**	**GR484**	**KWSX91**	**KKU1116**	**KKU2901**	**TSW**
NSW	**4.00**	0.08	0.12	-0.09	-0.10	-0.09	0.07	0.05	-0.04
BW	4.30	**4.28**	0.02	0.04	0.00	-0.14	-0.04	0.03	0.01
WPP	4.70	4.66	**5.36**	0.05	-0.16	0.07	-0.13	0.03	0.01
PEK	4.09	4.28	4.65	**4.09**	0.03	0.00	0.04	-0.02	-0.05
GR484	3.95	4.10	4.31	4.09	**4.11**	-0.02	0.00	0.09	0.15
KWSX91	4.09	4.10	4.68	4.20	4.05	**4.25**	0.18	0.02	-0.02
KKU1116	4.24	4.19	4.46	4.23	4.06	4.38	**4.14**	-0.13	0.02
KKU2901	4.15	4.19	4.55	4.09	4.08	4.14	3.98	**3.94**	-0.08
TSW	3.93	4.04	4.40	3.93	4.00	3.96	4.00	3.83	**3.51**
h_i	0.08	0.00	-0.18	0.05	-0.10	-0.03	0.02	0.04	0.12

Double cross combination mean = 4.20 cm, Parental mean = 4.19 cm, Average heterosis = 0.01 cm.

$LSD_{0.05}$ and $LSD_{0.01}$ for ear width of double cross combinations and parent hybrids are 0.23 and 0.32 cm, respectively.

S.E.s_{ij} = 0.18, S.E. h_i = 0.16

Table 28 Number of seed row of 36 double crosses (below diagonal) 6 parent hybrids (on diagonal) and specific heterosis effects (s_{ij}) of double crosses (above diagonal).

Variety	**NSW**	**BW**	**WPP**	**PEK**	**GR484**	**KWSX91**	**KKU1116**	**KKU2901**	**TSW**
NSW	**13.20**	0.53	0.65	-0.20	-0.44	-0.09	0.32	-0.24	-0.55
BW	13.45	**12.65**	-0.24	0.34	0.25	0.10	-0.55	-0.06	-0.37
WPP	15.25	13.63	**16.50**	-0.19	-0.13	0.07	-0.48	-0.14	0.45
PEK	13.10	12.90	14.05	**12.90**	0.07	-0.28	0.03	0.29	-0.05
GR484	12.65	12.60	13.90	12.80	**12.90**	-0.17	0.14	0.43	-0.14
KWSX91	13.25	12.70	14.35	12.70	12.60	**13.30**	-0.01	0.08	0.31
KKU1116	14.25	12.65	14.40	13.60	13.50	13.60	**14.10**	-0.07	0.62
KKU2901	12.70	12.15	13.75	12.88	12.80	12.70	13.15	**12.50**	-0.29
TSW	13.50	12.95	15.45	13.65	13.35	14.05	14.95	13.05	**13.20**
h_i	0.25	-0.2	-0.45	0.05	-0.16	-0.11	0.08	-0.11	0.65

Double cross combination mean = 13.4 rows, Parental mean = 13.5 rows

LSD $_{0.05}$ and LSD$_{0.01}$ for seed row number of double cross combinations and parent hybrids are 0.87 and 1.15 rows, respectively.

S.E.s_{ij} = 0.7 S.E.h_i = 0.6

5. Genetic effect for Agronomic characteristics base on analyses II and III

5.1 Combined analysis of variance for genetic effect

Analysis of variance across environments, based on Gardner and Eberhart model analysis II and III indicated that variance of variety effects (v_i), heterosis effect (h_{ij}) and GCA effects (g_i) of plant height (PH), ear height (EH), and tassel date (TAD) exhibited highly significant difference (p<0.01) (Table 29) and was significant (p<0.05) for silking date (SID). The h_{ij} was divided into average heterosis ($\hbar$), variety heterosis (h_i) and specific heterosis (s_{ij}); $\hbar$ was significant (p<0.05) in EH and TAD, h_i was significant (p<0.05) in PH and EH, and s_{ij} was significant (p<0.05) in TAD and SID (Table 29).

Environment x population interaction of PH, EH, TAD and SID exhibited highly significant difference (p<0.01). PH, EH, TAD and SID exhibited highly significant effects for interactions of environments with variety effect (v_i). PH and EH exhibited highly significant effects for interactions of environments with h_{ij} but TAD and SID did not show any significance. The Env x h_{ij} interaction of PH and EH divided into ENV x $\hbar$, ENV x h_i and ENV x s_{ij} interaction found that all interactions exhibited significant effects (p<0.05) (Table 29).

5.2 Genetic parameter effects across four environments

Average PH and EH of the hybrid varieties was 140 and 69 cm, respectively. PH and EH of GR484 were 163 and 78 cm, respectively (Table 5) and showed the highest estimate of v_i but their h_i were the lowest estimate (Table 30). KWSX91 and TSW showed positive effect on v_i, g_i and h_i for PH and EH. Average TAD and SID of the hybrid varieties were 48 and 49 days after planting respectively (Table 30). BW was late variety and showed high positive g_i for TAD and SID. While WPP was early variety and showed high negative g_i for TAD and SID. TSW showed high GCA effect and heterosis for PH, EH, TAD and SID.

Table 29 Mean square for agronomic traits from combined analysis of variance of genetic effect tested in 4 environments.

SOV	df	MS			
		PH[1]	EH	TAD	SID
Hybrids and Crosses (HC)	44	1288.65 **[2]	392.90 **	6.57 **	6.42 **
Varieties (v_i)	8	5584.20 **	1412.49 **	23.93 **	22.72 **
Heterosis (h_{ij})	36	334.09 **	166.33 **	2.71 **	2.80 *
Average ($\hbar$)	1	2955.53 ^{ns}	2023.51 *	12.47 *	11.20 ^{ns}
Variety (h_i)	8	489.58 *	207.66 *	3.45 ^{ns}	3.58 ^{ns}
Specific (s_{ij})	27	190.92 ^{ns}	85.30 ^{ns}	2.13 *	2.26 *
GCA (g_i)	8	4254.19 **	1283.14 **	17.65 **	15.64 *
Env x HC	132	176.02 **	92.95 **	1.83 **	2.24 **
Env x v_i	24	354.02 **	172.85 **	4.23 **	4.71 **
Env x h_{ij}	108	136.47 **	75.20 **	1.30 ^{ns}	1.69 ^{ns}
Env x $\hbar$	3	298.47 *	179.74 *	0.91 ^{ns}	2.07 ^{ns}
Env x h_i	24	156.82 *	80.69 *	1.97 ^{ns}	2.77 ^{ns}
Env x s_{ij}	81	124.44 *	69.70 *	1.11 ^{ns}	1.36 ^{ns}
E x g_i	24	170.97 **	84.25 ^{ns}	3.20 **	4.36 **
Pooled Error	212	89.50	48.17	1.02	1.31
Total	431				
CV(%)		6.62	9.68	2.12	2.32

[1] PH = plant height, EH = ear height, TAD = tassel date, and SID = silking date

[2] ^{ns} = not significant differentce, * and ** = Significant at 0.05 and 0.01 probability level, respectively.

Table 30 Variety effects (v_i), GCA effects (g_i) and variety heterosis (h_i) of parental waxy corn hybrid parents and correlation between variety effects and GCA effects ($r_{(vi,gi)}$) and variety heterosis ($r_{(vi,hi)}$) on agronomic traits.

Variety	**PH[1/] (cm)**			**EH (cm)**			**TAD (day)**			**SID (cm)**		
	v_i	g_i	h_i	v_i	g_i	h_i	v_i	g_i	h_i	v_i	g_i	h_i
NSW	12.25	11.81	5.69	-0.51	4.67	4.93	0.36	0.11	-0.08	0.08	-0.04	-0.09
BW	-9.00	-0.81	3.69	-5.64	-0.88	1.94	2.36	0.93	-0.25	2.71	0.85	-0.50
WPP	-16.63	-11.19	-2.87	-6.89	-6.83	-3.38	-0.89	-0.66	-0.22	-1.29	-0.72	-0.08
PEK	9.38	2.46	-2.23	6.86	1.94	-1.49	-0.39	-0.32	-0.13	-0.42	-0.38	-0.17
GR484	22.88	2.44	-9.00	9.11	0.78	-3.77	0.49	0.11	-0.14	0.46	0.10	-0.13
KWSX91	11.63	8.63	2.82	5.74	5.41	2.54	0.36	-0.39	-0.58	0.21	-0.22	-0.33
KKU1116	-16.13	-11.99	-3.93	-9.26	-7.88	-3.25	-1.39	-0.59	0.10	-0.92	-0.35	0.11
KKU2901	-17.25	-8.13	0.49	-1.76	-1.31	-0.43	-0.89	0.07	0.51	-0.54	-0.04	0.23
TSW	2.88	6.78	5.34	2.36	4.09	2.90	-0.01	0.77	0.77	-0.29	0.81	0.96
Mean	140.13	147.29	7.16	68.76	74.69	5.93	47.76	47.30	-0.47	49.42	48.98	-0.44
F-test	**[2/]	**	*	**	**	*	**	**	ns	**	*	ns
S.E.		4.01										
$r_{(vi,gi)}$		0.50			0.74			0.39			0.28	
$r_{(vi,hi)}$		-0.07			0.11			-0.34			-0.47	

[1/] PH = plant height, EH = ear height, TAD = tassel date, SID = silking date

[2/] ^{ns} = not significant differentce, * and ** = significant at 0.05 and 0.01 probability level respectively,

S.E. = standard error of the mean effects.

6. Heterotic groups based on SCA

Successful hybrid corn breeding programs relied on improvement of the germplasm sustaining its variance. To develop hybrid parental lines was usually obtained from germplasm classified genetically divergent heterotic groups (Hallauer *et al*., 1988). Diallel cross designs have been widely used to evaluate the performance of crosses among inbred lines or populations. GCA and SCA were determined in order to assign genotypes to heterotic groups (Hallauer and Miranda, 1988; Melchinger and Gumber, 1998). Clustering was done through SCA matrix and UPGMA. Group classification of varieties resulted in less average of SCA members within group as compared to those between groups. From our result, UPGMA dendogram based on the SCA matrix (Figure 5) revealed that heterotic pattern among 9 hybrids could be classified into 2 clusters**.** Cluster I comprised 5 hybrids from which WPP and TSW were assigned into subgroup I-1, whereas KKU1116, KKU2901 and PEK were in subgroup I-2. Cluster II was divided to be 2 subgroups: NSW and GR484 in subgroup II-1 but BW and KWSX91 in subgroup II-2. The SCA average of DC that parents were in Cluster I and Cluster II was -0.21 and -0.36 ton/ha (Table 31) respectively but the SCA average of DC which parents were in different clusters was 0.21 ton/ha (Table 31). The positive SCA indicated different heterotic groups of parents (Revilla *et al*., 2002; Pswarayi and Vivek, 2008). UPGMA dendogram based on the SCA matrix arranged members to be group by the lowest mean value in the group. Double cross BW/WPP and WPP/PEK showed positive SCA effect while double cross BW/KWSX and PEK/KWSX showed negative SCA effect. Heterosis group classification, a line may sometime be assigned to be more one heterotic group, the largest negative value line was kept in the heterotic group (Fan *et al*., 2009)

Table 31 The SCA average of DC which parents were intra and inter cluster.

Intracluster I	
DC	**SCA**
WPP/PEK	0.40
WPP/KKU1116	-0.18
WPP/KKU2901	0.38
WPP/TSW	-1.14
PEK/KKU1116	-0.47
PEK/KKU2901	-0.63
PEK/TSW	0.20
KKU1116/KKU2901	-1.01
KKU1116/TSW	0.07
KKU2901/TSW	0.25
Average	-0.21

Intracluster II	
DC	**SCA**
NSW/BW	0.69
NSW/GR484	-0.66
NSW/KWSX91	-0.47
BW/GR484	-0.58
BW/KWSX91	-0.62
GR484/KWSX91	-0.50
Average	-0.36

Intercluster	
DC	**SCA**
NSW/WPP	-0.13
NSW/PEK	-0.33
NSW/KKU1116	0.03
NSW/KKU2901	0.55
NSW/TSW	0.31
BW/WPP	0.25
BW/PEK	1.04
BW/KKU1116	0.41
BW/KKU2901	-0.62
BW/TSW	-0.56
WPP/GR484	0.28
PEK/GR484	0.11
GR484/KKU1116	0.08
GR484/KKU2901	0.46
GR484/TSW	0.81
WPP/KWSX91	0.14
PEK/ KWSX91	-0.32
KWSX91/KKU1116	1.08
KWSX91/KKU2901	0.62
KWSX91/TSW	0.08
Average	0.21

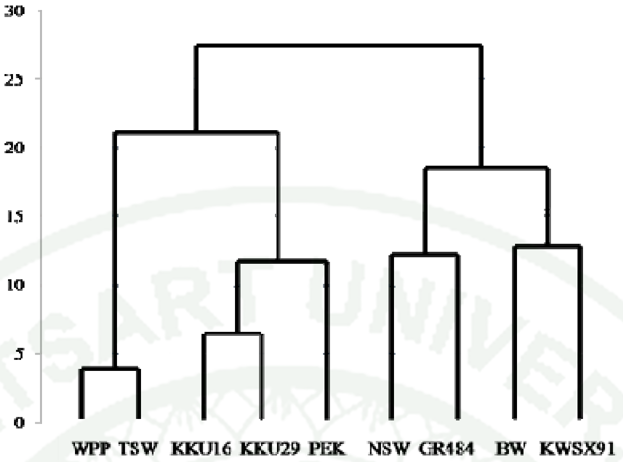

Figure 5 UPGMA dendogram of 9 hybrid parents based on SCA

7. Troyer's genetic distance

TGD based on MDY was significantly different, ranging from 0.40 between KKU1116 and KKU2901, up to 1.21 between KKU1116 and KWSX91. There were only two pairs of hybrid having TGD index lower than 0.5 while 24 pairs showed the TGD index higher than 0.75. The average index of MDY across the experiment was 0.83, indicating that waxy corn hybrids improved in Thailand had rather high diversity (Table 32). Theoretically, TGD value ranged from 0 to 1 depending on the percentage of relatedness of four parental inbreds of both hybrids (Troyer *et al*., 1988). TGD of KKU1116/KKU2901 and WPP/TSW were low with the values of 0.40 and 0.50 respectively. The parental lines of KKU1116/KKU2901 was developed from the same source and probably had parents in common or closely to their genetic constitution (Balestre *et al*., 2008), while those of WPP/TSW came from different sources. The parents of NSW/TSW, although originated from the same source, had high TGD of 1.05. Choukan *et al*. (2006) found that closely related inbred lines by pedigree showed high genetic diversity in different environments of selection. The genetic divergence among different germplasm sources was low; this might be due to continuous exchange of genetic materials. Ear appearance was an important trait to be focused in germplasm improvement. Different ear shapes and qualities of hybrids released from the same source were available upon consumer’s need in each location. Thus the TGD indices may not reveal source relationship. The companies usually emphasized on consuming quality and yield potential in their breeding objectives. It was possible that inbred lines used as parents of hybrids in each breeding program were different depending on their selection practices varying in consuming quality and agronomic traits. Our results were similar to the work of Phumichai *et al.* (2008) who inferred that TGD was not a good measure of diversity between the single cross parents. Parents from different sources had more genetic difference than parents originating from the same germplasm source. Jompuk *et al.* (2000) assessed TGD in field corn and concluded that hybrids of the same source showed high degree of relatedness, while hybrids of different sources had more diverse parents with quite high degree of relatedness. However, TGD index indicated that parental lines of TSW and WPP in subgroup I-1 (classified based on the SCA matrix) were closely related. WPP/TSW double cross combination was low in heterotic effect. KKU1116, KKU2901 and PEK in subgroup I-2 were closely related. Heterosis of double cross combinations, KKU1116/KKU2901, PEK/KKU1116 and PEK/KKU2901 were likewise low. The parental line of KWSX91 was distantly related with parental lines of the other hybrids. The relationship among the parental lines of hybrids was important to support plant breeder’s decision to sustain variation of his/her breeding population.

Table 32 Troyer’s genetic distance average across four environments (above diagonal) and mid parent heterosis (%) of double crosses (below diagonal), of 9 waxy corn hybrid varieties.

Variety	NSW	BW	WPP	PEK	GR484	KWSX91	KKU1116	KKU2901	TSW
NSW		1.20	0.88	0.88	0.70	1.10	0.80	1.04	1.05
BW	7.3		0.88	1.07	0.64	0.95	0.82	0.68	0.72
WPP	-6.0	-7.5		0.91	0.77	1.03	0.70	0.84	0.50
PEK	-7.0	2.5	-7.8		0.67	0.93	0.59	0.64	0.81
GR484	-18.8	-23.3	-16.8	-16.8		0.83	0.62	0.78	0.77
KWSX91	4.6	-4.8	1.5	-3.4	-13.9		1.21	1.16	1.05
KKU1116	-10.1	-11.8	-21.9	-22.8	-24.9	8.0		0.40	0.57
KKU2901	2.3	-18.7	-9.8	-19.9	-15.1	8.1	-33.1		0.78
TSW	0.2	-18.3	-29.5	-10.2	-11.0	1.9	-20.5	-11.9	

S.E.$_{(TGDij)}$ = 0.10

F-test: ENV. (ns), TGD (**), ENV x TGD (ns)

LSD$_{.05\ (TGD)}$ and LSD$_{.01(TGD)}$ for Troyer’s genetic distance of double cross combinations are 0.28 and 0.37 respectively.

8. Relationship between genetic distance and heterosis

The MDY performance was positively correlated with heterosis (%H) (r = 0.78**), SCA (r = 0.66**) and TGD (r = 0.77**), but no apparent correlation was detected between MDY and phenotypic diversity (PD) (Table 33). Highly positive correlation between heterosis and TGD (r = 0.98**) agreed with previous studies (Phumichai *et al.*, 2008). TGD and SCA showed moderately positive correlation (r = 0.62**). Genetic divergence among parents was required for high heterosis but phenotypic diversity could act as a poor predictor of high heterotic hybrid combinations. Phenotypic diversity provided only a rough estimate of the true relatedness between two lines and could discriminate their derived progenies poorly (Lee *et al*., 2007). Combinations of distance measured based on pedigree and phenotypic traits were useful to determine genetic distance, therefore genetic resource was very important to be considered for measuring phenotype. Jaccard similarity (JS) based on 20 SSR markers was poorly correlated with MDY (-0.11), SCA (0.05), TGD (-0.18), %H (-0.22) and PD (0.09) (Table 33). Correlation coefficient between them was not significant. Genetic distance based on DNA markers was a reliable tool in determining genetic relationships and diversity among genotypes but insufficient to be used as a reliable indicator for predicting hybrid yield and yield heterosis. (Xangsayasane *et al*., 2010) The correlation of the genetic distances between Roger’s modified distance (RMD) inter-group hybrids with SCA was small and significant. In contrast, there was no association between intra-group hybrids and their genetic distances (Balestre *et al.*, 2008). Linear relationship of morphological distance on pedigree distance in spring wheat gave R^2 = 0.46 (p<0.01), when the entire range of pedigree distances was systematically sampled (Van Beuningen and Busch, 1997).

Table 33 Correlation coefficient (*r*) among marketable dehusk yield (MDY), specific combining ability (SCA), heterosis (H), Troyer's genetic distance (TGD), phenotypic diversity (PD) and Jaccard similarity (JS) in double cross combinations

	MDY	**SCA**	**TGD**	**H**	**PD**
SCA	0.66**[1/]				
TGD	0.77**	0.62**			
H	0.78**	0.63**	0.98**		
PD	0.25^{ns}	0.24^{ns}	0.00^{ns}	0.00^{ns}	
JS	-0.11^{ns}	0.05^{ns}	-0.18^{ns}	-0.22^{ns}	0.09^{ns}

[1/] ^{ns} = not significant differentce,

* and ** = Significant at 0.05 and 0.01 probability level, respectively.

Conclusion

The results obtained from this study can be concluded as the followings:

1. Waxy corn hybrids improved in Thailand had diversity of genetic background. These varieties displayed potential to be used as germplasm for hybrid breeding program.

2. There was a positive correlation between degree of heterosis and Troyer's genetic distance (TGD). From this study, two distinct clusters were identified based on SCA. Cluster I comprised 5 hybrids (WPP, TSW, KKU1116, KKU2901 and PEK) while cluster II had 4 hybrids (NSW, GR484, BW and KWSX91). This relationship suggested that breeders can improve two separate sets through reciprocal recurrent selection method. Advanced farmers desiring to keep their own population for reducing seed cost can produce double cross population from some single cross hybrids such as between BW and PEK.

3. There was no correlation between phenotypic and genotypic group estimated by Jaccard similarity based on 20 SSR markers.

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