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Study on Heterosis and Genetic Distance of S_6 Inbred Lines of Maize

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ABSTRACT

Objectives of this research were to find out the heterosis value and genetic distance on several inbred lines and to find out the relationship between the genetic distance and heterosis in maize. The research performed in February to November 2011, which comprised of 2 researches: molecular analysis and in the laboratory and the field trial. The molecular analysis on inbred lines, which would be used as parents, was performed in Biotechnology Laboratory of Agro ecology Department, Faculty of Agriculture, University of Brawijaya, and Malang of Indonesia. The field trial was conducted in Kajang, Junrejo, Batu. Materials of the laboratory research included 35 genotypes of maize, which comprised of 33 inbred lines of the sixth-generation of selection (S_6), and as comparison, 2 open pollinated varieties were applied: Bisma and Lamuru. Steps for molecular analysis at the laboratory were as followed: (1) DNA isolation, (2) DNA-quality test, (3) PCR SSR, (4) Visualization of the amplification result. Materials for the field experiment included: 140 F_1 hybrids, 28 lines, 5 testers was planted using Randomized Complete Block Design (RCBD) with 2 replications. Observation was taken on 9 agronomic characters of maize: the plant height (cm), leaf length (cm), days pollen to shed (day), ear weight plant^{-1} (kg), kernel weight plant^{-1} (kg), diameter of ear (cm), number of rows ear^{-1}, and kernel water content (%), as well as the grain (kg ha^{-1}). Results of the research showed that there were any interaction of Line x Tester in all characters except for leaf length and kernel water content. The estimate values of specific combining ability (SCA) and the heterosis value were varied among F1 hybrid and among of the observed characters. The value of genotypic correlation among the characters were ranged 0.20 – 1. The genetic distance between parents of the F_1 hybrid was ranged from 0.25 to 0.65. Correlation coefficient of Spearman's Rank between the genetic distance and SCA ranged -0.009 – 0.143, correlation coefficient of Spearman's Rank between the genetic distance and heterosis ranged -0.120 – 0.181.

Keywords: *Genetic Distance, heterosis, molecular, phenotype, maize*

INTRODUCTION

Heterosis is the reverse of inbreeding depression, in which the plant regained better appearance as shown by F_1 hybrid having better phenotype character appearance than mean of both the parents [1]. Ruswandi [2] described that combining ability was a relative ability of an inbred line when it was crossed with other inbred line in order to create novel characters as desired. Information of general combining ability (GCA) played significant role in inbred evaluation, while the specific combining ability (SCA) played significant role in determining the best F_1 hybrid on maize hybrid development.

Molecular marker technology was effective in identification and genotypic mapping on maize, as well as in learning phenomena as heterosis and genotype, as well as their interaction with environment. The application of molecular marker would also improve accuracy and efficiency in maize breeding [3][4]. The genetic distance-based molecular marker can be applied for initial grouping based on the heterosistic pattern [5]. The application of SSR molecular marker to obtain information about genetic distance among those inbred lines could be used to find out the heterosistic group without any influences by the tester, as well as the genotype interaction with environment and it did not require any crossing of breeding [6].

Estimation of the genetic relationship among parents was useful in the planning of crossing for hybrid [7]. The genetic relationship pattern of the inbred lines, which was based on the morphological characters and the yield components, could be used to determine the inbred lines, which were used to arrange novel hybrid that having higher yield and heterosis. The greater genetic relationship pattern of the two inbred

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lines was crossed, the greater possibility of the heterosis existed. In relation to this, further study on genetic relationship is required in order to find out the genetic distance among the inbred lines, which would be applied in developing hybrid varieties. The molecular marker can be used to predict heterosis [4][6][8].

MATERIALS AND METHODS

Genetic Material

Molecular analysis applied 35 genotypes of maize, which comprised of 33 inbred lines on the sixth-generation of selection (S_6) and as comparison, 2 open pollinated varieties were applied: Bisma and Lamuru. Materials of the field experiment included: 28 inbred lines, 5 testers, and 140 F_1 hybrids.

Molecular Analysis

Samples of the molecular analysis to observe the genetic distance among inbred lines used young leaves, 25-35 days after planting. Numbers of the genotype^{-1} samples were 10 plants. DNA isolation was applied using CTAB method [9][10], which was modified using an active carbon [11]. Measurement of the DNA quality used electrophoresis, in which 1% agarose was dissolved in TBE 0.5x. 0.4 g of agarose was dissolved in 40 ml TBE and poured into the plate and allow it to be hard. Then, the gel submerged in the electrophoresis chamber. The sample used 2 µl DNA and 1 µl loading dye, which were put into wells. The electrophoresis was performed using 100 volt voltage for about half an hour. Result of such electrophoresis was visualized over the ultraviolet light.

PCR reaction optimization program was performed before amplification. Optimization was performed to obtain PCR optimum condition for DNA amplification using primer microsatellite, which had been previously determined. Also, optimization was performed to choose primer, which had higher polymorphism. PCR reaction optimization program was: 1 initial denaturation cycle under temperature of 94 ^{o} C for 4 minutes, followed by 37 denaturation cycles under temperature of 94 ^{o} C for 50 seconds and annealing temperature of 55 ^{o} C for 1 minute. PCR cycle was completed by 1 final extension cycle under temperature of 72 ^{o}C for 1 minute. This optimization used 9 pairs of SSR primers as presented in the table as followed: P-umc1165, Nc030, Umc1294, ZTC161, Phi001, Phi034, Phi057, Phi080 and Phi119[12].

Electrophoresis was applied to find out result of the DNA amplification using PCR. One percent of agarose was dissolved in 40 ml buffer TBE 0.5 xs, then it was electrophoresed under 100 V voltages for 30 hours. Then, the gel was submerged in Et Br (Ethidium Bromide) for 15 minutes. The visualization was performed over the ultraviolet light.

Field Experiment

Treatment in the experiment included: 28 inbred lines, 5 testers and 140 F_1 hybrids, which were planted using Randomized Complete Block Design with 2 replications. The planting was performed a week later after the soil had been cultivated. Size of the plot was 1.4 meter x 2.5 meter, distance between plots was 0.70 meter. The planting distance between rows was 0.70 m and 18 cm between lines. 20 plants were taken as sample in each replication.

Applications of fertilizers dosage were 300 kg NPK/Ha and 100 kg Urea. Application of the fertilizers was given 4 times in different period as follow : (1) Application of fertilizer 1, was given during the initial planting (0 day after planting), for about 100 kg NPK/Ha, (2) Application of fertilizer 2, was given within 21 days after planting, for about 75 kg NPK/Ha and 50 kg Urea/Ha, (3) Application of fertilizer 3, was given within 45 days after planting, for about 75 kg NPK/Ha and 25 kg Urea/Ha, (4) Application of fertilizer 4, was given within 60 days after planting, for about 50 kg NPK/Ha and 25 kg Urea/Ha.

Observation was taken on 9 agronomic characters of the maize: the plant height (cm), leaf length (cm), days to pollen shed (day), ear Weight plant^{-1} (kg), kernel weight plant^{-1} (kg), diameter of the ear (cm), number of rows ear^{-1}, and water content (%), as well as the grain yield (kg^{-ha}).

Data Analysis

Experiment in the Laboratory. Data obtained from photo-documentation was DNA band pattern as a result of PCR amplification using primers SSR. DNA band resulted from amplification was interpreted as qualitative data by observing the presence and absence of such DNA band resulted from amplification, which was transferred to binary data through scoring on one position of the equivalent band row. Scoring was done by: if there was any band, the score was one (1) and if there was no band, the score was zero (0). Then, this data was used to calculate genetic similarity coefficient and to construct dendogram.

Genetic relationship was determined by Jaccard's similarity coefficient with the formula by Sneath and Sokal (1973) in [13]:

$Sj = A/(A+B+C)$……(1)

Note:
Sj: Jaccard's similarity coefficient,

- A: numbers of the DNA band (*alil*), which belong to both genotype 1 and 2
- B: numbers of specific band belongs to genotype 1,
- C: number of specific band belongs to genotype 2.

The genetic similarity was analyzed using NTSYSpc (Numerical Taxonomic System) program version 2.02i. Matrix analysis for the genetic distance was obtained from result of the genetic similarity analysis by the formula by Nie and Lie (1979) in [14]:

$GD = 1 - S$ ……(2)

where:

- GD = genetic distance
- S = Similarity in genetic.

Field Data. The linear additive model for Line x tester analysis including the parents was as followed:

$Y_{ijklt} = \mu + T_i + T_iC_j + G_l + H_t + (GH)_{lt} + \sum_{ijklt}$…(3)

whereas:

- Y_{ijklt} = the observation value for parents-*i*, parents *vs* crosses-*ij*, line-*l*, testers-*t*, interaction-*lt* and replications-*k*
- μ = general median value
- T_i = influence of the parents-*I*
- T_iC_j = influence of the parents *vs* crosses-*ij*,
- G_l = influence of line-*l*
- H_t = influence of the testers-*t*
- $(GH)_{lt}$ = the interaction influence of GH on G-*l* and H-*I*
- $\sum_{ijklt}$ = the experiment error for the parents-*i*, parents *vs* crosses-*ij*, genotypes-*l*, testers-*t*, interaction-*lt* and replications-*k* [15]. Influence of the specific combining ability was estimated using formula of Singh and Chaudhary [15] as followed:

$Sij = ((Xij./r) - (Xi../tr) - (X.j.\ l\ gr)) + (X…/\ l\ gtr))$……..(4)

Note:

- l = lines,
- t = testers,
- r = replications.

Estimation of the heterosis value was based on the mean parent (H%) = [(F1-MP)/MP x 100]…….(5)

where:

- MP: mean parents (P1+P2)/2; in which P1: first mean parent, P2: second mean parent.

Close genetic relationship among the observed characters was analyzed using simple genotypic correlation analysis of Singh and Chaundary [15].

Formula for genotypic correlation coefficient between two agronomic characters was:

$rg(x_1\ x_2) = Cov\ (x_1.x_2)/\sqrt{\ } \sigma^2(x_1).\ \sigma^2(x_2)$…….(6)

where:

- rg= genetic correlation coefficient between character x_1 and x_2
- Cov $(x_1.x_2)$ = covariance between character x_1 and x_2
- $\sigma^2(x_1)$ = diversity of x_1 and $\sigma^2(x_2)$ = diversity of x_2.

Correlation Analysis between Molecular Data and Phenotype Data

Relationship between phenotype and molecular data was analyzed using correlation coefficient of Spearman [15][16] by the formula as followed:

$r_s = 1 - [(6d_i^2)/n(n^2-1)]$……..(7)

where:

- r_s = correlation coefficient value of Spearman's Rank
- d = difference between ranking on the first and the second data
- n = number of treatment

RESULTS AND DISCUSSION

Line x Tester Analysis and Specific Combining Ability

Result of the variance analysis on genotype showed that all characters had significant influence. Parent x crosses showed the significant influence on all characters except the water content in harvest time

character. Crosses showed significant influence on all characters except the leaf length character. Lines showed significant influence on all of the observed characters. Testers showed significant influence on all characters except the leaf length. There was an interaction of Line x tester on all characters except the leaf length and water content in the harvest time (Table 1). Result of this research was conformed to research performed by Mosa [17], Motawal [18] and Sundararajan [19], in which the significance was observed on line, tester, line x tester.

Table 1 Analysis of line x tester on several observed characters: Leaf Length (LL), days pollen to shed (DPS), Ear weight plant^{-1} (EWP), kernel weight plant^{-1} (KWP), diameter of the ear (DE), number of rows ear^{-1} (NRE), Water content in the harvest time (WC) and grain yield.

Source of variation	Mean sum of Squares								
	D.F	LL	DPS	EWP	KWP	DE	NRE	WC	Grain yield
Replications	1	174.8	0.3	0.01533*	0.0094*	0.03	0.3	0.3	18033219.8*
Genotypes	172	115.8*	32.5*	0.0034*	0.0020*	0.20*	3.3*	4.5*	11934357.1*
Parents	32	195.1*	55.9*	0.0016*	0.0009*	0.20*	4.0*	7.5*	7485856.5*
Parents vs. Crosses	1	3107.5*	153.5*	0.2334*	0.1567*	8.57*	4.0*	7.4	1069162835.3*
Crosses	139	76.1	26.2*	0.0022*	0.0012*	0.15*	3.1*	3.8*	5352512.1*
Lines	27	147.0*	47.2*	0.0018*	0.0010*	0.16*	5.3*	6.5*	6538253.8*
Testers	4	148.3	392.3*	0.0447*	0.0223*	1.06*	22.2*	28.8*	33150531.4*
Lines x Tester	108	55.6	7.4*	0.0007*	0.0004*	0.11*	1.9*	2.2	4026520.4*
Error	172	87.7	1.5	0.0004	0.0002	0.08	1	2.5	1144548

Note: * significant at P<0.05 level of significance.

Result of the observation on all genotypes showed variations in phenotype character appearances in plants. Leaf length ranged 59.5 to 108.3 cm, and the mean was 86.7 cm. Days pollen to shed ranged 41.2 to 66.2 days, and the mean was 59.7 days. Ear weight plant^{-1} ranged 0.08 to 0.29 kg, and the mean was 0.20 kg. Kernel weight plant^{-1} ranged 0.06 to 0.22 kg, and the mean was 0.15 kg. Diameter of ear ranged 3.5 to 5.5 cm, and the mean was 4.4 cm. Number of rows ear ranged 9.7 to 16.7, and the mean was 13.5. Kernel water content in the harvest time ranged 12.1 to 22.4%, and the mean was 19.1%. The grain yield ranged 3324.3 kg ha^{-1} to 15403.7 kg ha^{-1}, and the mean was 10683.7 kg ha^{-1}.

Nine F_1 hybrids showed the highest results: G-49XG-T37 (13751.9 kg ha^{-1}), G-36XG-T22 (14480.8 kg ha^{-1}), G-17XG-T22 (14610.8 kg ha^{-1}), G-38XG-T22 (14664.5 kg ha^{-1}), G-35XG-T22 (14672.5 kg ha^{-1}), G-B0XG-T22 (14711.0 kg ha^{-1}), G-49XG-T22 (15769.5 kg ha^{-1}), G-51XG-T22 (14797.6 kg ha^{-1}) and G-06XG-T15 (15403.7 kg ha^{-1}). The grain yield was become the main focus in plant breeding, due to in general, the grain yield had connected to other characters [20].

Specific combining ability (sca) analysis showed variation among the observed characters, by positive and negative values. Result of SCA analysis is presented in Table 2.

Table 2 The range of Specific cability effects (sca) and standard error for 8 characters of maize

Characters of maize	Range of sca	Standard Error
Leaf Length	-12.2 to 18.4	6.60
Days pollen to shed	-4.3 to 5.5	0.90
Ear weight plant^{-1}	-0.05 to 0.04	0.01
Kernels weight plant^{-1}	-0.03 to 0.03	0.01
Diameter of Ear	-0.7 to 0.8	0.20
Number of kernels rows cob^{-1}	-2.4 to 2.6	0.70
Kernel water content in the harvest time	-2.6 to 1.8	1.10
Grain yield	-3682.2 to 5251.7	756.5

SCA value showed variation among the observed characters, such as: leaf length ranged -12.2 (G-01XG-T00) to 18.4 (G-06XG-T14), days pollen to shed ranged -4.3 (G-46XG-T37) to 5.5 (G-46XG-T14), ear weight ranged -0.05 (G-03XG-T15) to 0.04 (G-46XG-T14, G-06XG-T14, G-20XG-T37), kernel weight plant^{-1} ranged -0.03 (G-20XG-T00, G-46XG-T00, G-03XG-T15, G-42XG-T22, G-23XG-T22) to 0.03 (G-46XG-T14, G-20XG-T37), diameter of ear ranged -0.7 (G-01XG-T15) to 0.8 (G-13XG-T15), number of rows ear^{-1} ranged -2.4 (G-18XG-T14) to 2.6 (G-18XG-T22), kernel water content in the harvest time ranged -2.6 (G-36XG-T22) to 1.8 (G-49XG-T00, G-49XG-T37) and the grain yield ranged -3682.2 (G-02XG-T15) to 5251.7 (G-46XG-T14).

8 lines (G-02, G-04, G-11, G-18, G-19, G-34, G-40 and G-B0) showed significant SCA and had positive values when they were crossed with tester GT-00. 6 lines (G-01, G-02, G-03, G-20, G-42 and G-46) showed significant SCA and had positive values when they were crossed with tester G-T14. 7 lines (G-

06, G-07, G-08, G-21, G-23, G-24 and G-26) showed significant SCA and had positive values when they were crossed with tester G-T15. 9 lines (G-01, G-08, G-17, G-35, G-36, G-38, G-40, G-51 and G-B0) showed significant SCA and had positive values when they were crossed with tester G-T122. 4 lines (G-01, G-18, G-24 and G-26) showed significant SCA and had positive values when they were crossed with tester G-T37. The influence of SCA could bring about both positive and negative values. Positive value meant that F_1 hybrid was better than F_1 hybrid that having negative value (on the equivalent character) [2][18].

Heterosis

The heterosis value varied between F_1 hybrid and among the observed characters. In this study, the heterosis was calculated according to the standard heterosis value or mid parent heterosis (MPH), the mean value between both mean parents.

Table 3 The range of standard heterosis for 8 characters in maize.

Traits	Range of standard heterosis
Leaf Length	-11.1 to 41.7
Days pollen to shed	-11.9 to 28.9
Ear weight plant^{-1}	-1.0 to 133.6
Kernels weight plant^{-1}	6.2 to 131.0
Diameter of ear	-8.0 to 25.5
Number of rows ear^{-1}	-21.8 to 22.5
Kernel water content in the harvest time	-19.8 to 30.5
Grain yield	-1.7 to 212.4

Heterosis varied among characters of maize. Heterosis value for leaf length character ranged -11.1 (G-44XG-T14) to 41.8 (G-26XG-T15), days pollen to shed ranged -11.9 (G-26XG-T37) to 28.9 (G-40XG-T37), ear weight ranged -1,0 (G-06XG-T37) to 113.6 (G-26XG-T22), kernel weight plant^{-1} ranged 6.2 (G-06XG-T37) to 131.0 (G-26XG-T22), diameter of ear ranged -8.0 (G-01XG-T15) to 25.5 (G-B0XG-T00), number of rows ear^{-1} ranged -21.8 (G-03XG-T22) to 22.5 (G-06XG-T14), kernel water content in the harvest time ranged -19.8 (G-49XG-T15) to 30.5 (G-26XG-T14) and the grain yield ranged -1.7 (G-24XG-T14) to 212.4 (G-38XG-T00).

Several F_1 hybrid showed higher heterosis on the grain yield character, such as: G-38XG-T00 (212.4%), G-02XG-T00 (201.9%), G-26XG-T00 (189.0%), G-B0XG-T00 (167.4%), G-03XG-T00 (147.5%), G-38XG-T15 (130.4%), G-38XG-T22 (134.1%), G-26XG-T37 (102.9%), G-38XG-T14 (103.9) and some other F_1 hybrid. Mean value of heterosis varied among the used testers, such as: G-T00 (106.2%), G-T14 (42,0%), G-T15 (62.7%), G-22 (62.5%) and G-T37 (54.35). Heterosis was a character comparison on inbred line and its F_1 hybrid [21][22].

Genetic Correlation among the Observed Characters

Data showed genetic correlation between the grain yield and the leaf length character (0.95), ear weight plant^{-1} (0.88), kernel weight plant $^{-1}$ and diameter of ear (0.93). Kernel water content correlated to the leaf length character (0.57), days to pollen shed (0.65), ear weight plant^{-1} (0.55), kernel weight plant^{-1} (0.56), diameter of ear (0.61), and number of rows ear^{-1} (0.90). Number of rows ear correlated to days pollen to shed (0.61), ear weight plant^{-1} (0.54), kernel weight plant^{-1} (0.52) and diameter of ear (0.73). Diameter of ear correlated to leaf length (0.90), ear weight plant^{-1} (1,00) and kernel weight plant^{-1} (1,00). Kernel weight plant^{-1} correlated to leaf length (0.89) and ear weight plant^{-1} (0.99). Ear weight plant^{-1} correlated to leaf length (0.88). Results of other researches were comparable to result of this research, in which there was significant and positive correlation among characters of maize [23][24][25].

Table 4 Genetic correlation among characters of maize

Maize characters	1	2	3	4	5	6	7	8
Leaf length (1)	1.00	0.26	0.88*	0.89*	0.90*	0.36	0.57*	0.95*
Days to pollen shed (2)		1.00	0.36	0.33	0.40	0.61*	0.65*	0.20
Ear weight plant^{-1} (3)			1.00	0.99*	1.00*	0.54*	0.55*	0.88*
Kernel weight plant^{-1} (4)				1.00	1.00*	0.52*	0.56*	0.90*
Diameter of ear (5)					1.00	0.73*	0.61*	0.93*
Number of rows ear^{-1}(6)						1.00	0.90*	0.48
Kernel water content in the harvest time (7)							1.00	0.48
Grain yield (8)								1.00

Genetic Similarity and Genetic Distance (GD)

Three out of 9 primers, which were used, had higher polymorphism, such as: umc1294, ZCT161 and phi080. Senior [26] applied the microsatellite marker in maize and it showed higher polymorphism. Those three primers were used to estimate the genetic distance of 33 inbred lines and 2 open pollinated varieties (OPV). Result of the dendogram analysis showed that 35 genotypes of maize were divided into 2 main clusters, A and B, in which the similarity level was 35%. Cluster A comprised of 33 genotypes and cluster B comprised of 2 genotypes. Cluster A was divided into 2 sub-clusters, such as: A_1 and A_2. Sub-cluster A_1 comprised of 11 genotypes, while sub-cluster A_2 comprised of 22 genotypes. (Figure 1)

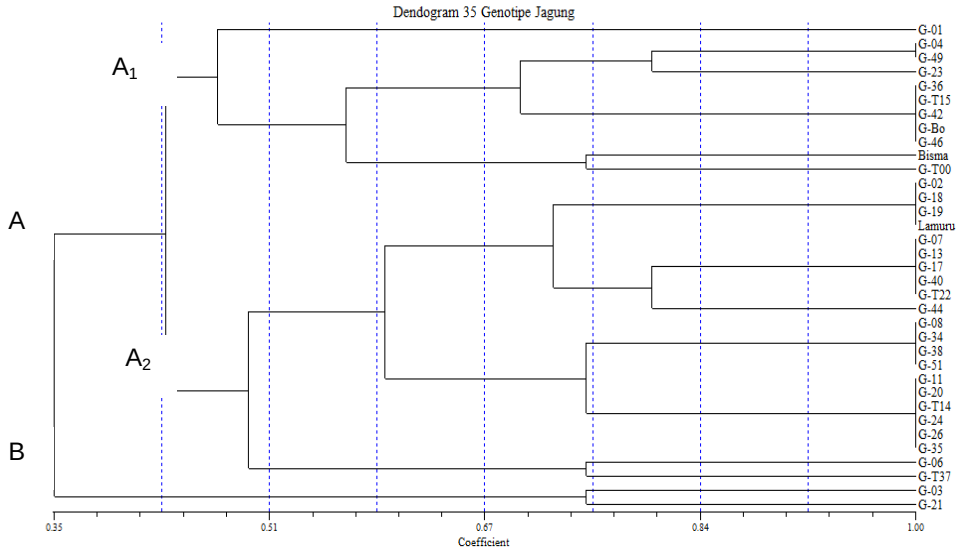

Figure 1 Dendogram of 35 genotypes of maize based on SSR marker using UPGMA method and constructed based on Jaccard's similarity coefficient.

The genetic distance calculation was based on genetic similarity. Genetic distance (GD) varied among F_1 hybrids, which ranged 0.25 (G-G-11XG-T14, 24XG-T14, G-26XG-T14, G-35XG-T14, G-36XG-T15, G-42XG-T15, G-46XG-T15, G-07XG-T22, G-13XG-T22, G-17XG-T22, G-40XG-T22) to 0.65 (G-07XG-T00, G-21XG-T00, G-03XG-T14, G-21XG-T14, G-03XG-T22, G-21XG-T22, G-03XG-T37, G-T21XG-T37). Several F_1 hybrids had lower genetic distance and the other had some F_1 hybrid, which had higher genetic distance.

Relationship between Genetic Distance and Heterosis

Correlation between the genetic distance and SCA as well as the heterosis was using Spearman's rank correlation. Correlation coefficient of Spearman's Rank between the genetic distance and SCA ranged -0.009 – 0.143, correlation coefficient of Spearman's Rank between the genetic distance and heterosis ranged -0.120 – 0.181.

Table 10 Correlation coefficient of Spearman's Rank (r_s) and SCA as well as the genetic distance on the plant characters

Characters of maize	**r_s**	
	SCA	Heterosis
Leaf length	0.133	0.144
Days to pollen shed	0.036	-0.120
Ear weight plant^{-1}	0.125	0.102
Kernel weight plant^{-1}	-0.009	0.110
Diameter of ear	0.013	0.033
Number of rows ear	-0.005	0.049
Kernel water content at harvest time	-0.099	0.042
Grain yield	0.143	0.181*

Note: * significant at p <0.05 level of significance.

Result of the correlation analysis showed positive relationship between genetic distance and SCA on characters of leaf length (0.133), ear weight plant^{-1} (0.125) and grain yield (0.143). Significant correlation

was found between heterosis and the grain yield. Positive correlation was found between heterosis and leaf length (0.144), ear weight plant^{-1} (0.102), and kernel weight plant^{-1} (0.110). Relationship between the genetic distance and heterosis in the grain yield was presented by simple linear regression as followed:

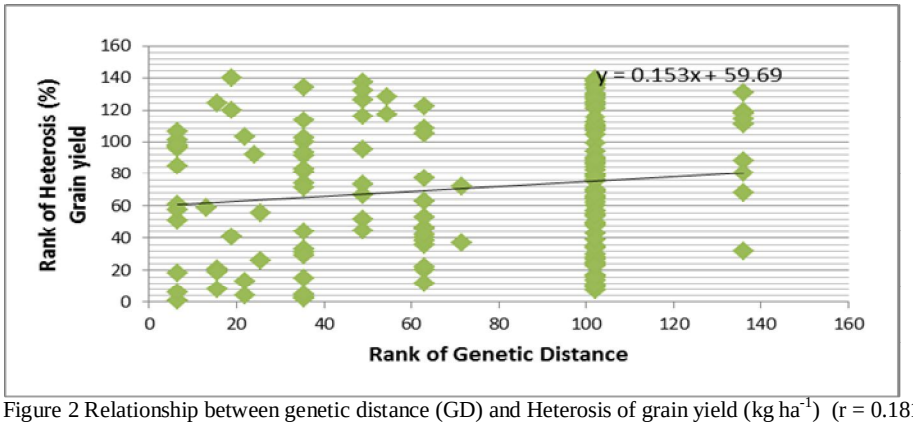

Figure 2 Relationship between genetic distance (GD) and Heterosis of grain yield (kg ha^{-1}) (r = 0.181, using Spearman's rank correlation coefficient)

Figure 2 showed formula y = 0.153x + 59.69, which indicated that the greater genetic distance, the higher value of heterosis on the grain yield. The application of molecular SSR in order to gain information about genetic distance among the inbred lines, can be used to find out the heterosisic group without any influenced by tester, as well as the genotype interaction with the environment, and any crossing was unnecessary [6][27]. The genetic distance, which based on SSR marker, showed positive correlation with the result of F_1 hybrids and the standard heterosis [4]. Relationship between genetic distance and heterosis could be used to predict F_1 hybrid appearance [28].

CONCLUSION

Conclusions of the research are (1) there was different values for SCA and heterosis on F_1 hybrid, (2) there was significant genetic correlation among the phenotype characters of maize, (3) there was different values of genetic distance in F_1 hybrid based on the molecular marker, and (4) there was greater and significant correlation between the genetic distance and the grain yield, (5) The greater genetic distance on F_1 hybrid, the higher heterosis on the specific character in maize. It is suggested for further research to apply more primers in order to obtain more convincing and accurate results.

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REFERENCES

1. Brieger, F.G. 1950. The Basic of Heterosis in Maize. *J. Genetic* 35:420-427
2. Ruswandi,D., Anggia E.P., Tri Hastini, A. Suhada, N. Istifadah, A. Ismail, E. Suryadi, S. Ruswandi and N. Rostini. 2006. Selection on maize hybrid DR Unpad based on stability and adaptability of the result at eight locations in Indonesia. *Zuriat* 19 (1): 71-85
3. Stuber, C.W. 1995. Mapping and manipulating quantitative traits in maize. *Trends in Genetic*. 11: 477-481
4. Pongsai, C., X. Tan, A. Silapapun, P. Suthipong and L. Wei. 2009. The use of SSR markers to identify heterotic pattern of F1 hybrids in two tropical maize populations. *Sci. Technol*. 16: 175-184.
5. Dhliwayo, T., K. Pixley, A. Menkir, and M. Warburton. 2009. Combining Ability, Genetic Distances, and heterosis among Elit CIMMYT and IITA Tropical Maize Inbred Lines. *Crop Sci* 49: 1201-1210.

6. Aguilar, C.G., I. Schuster, A.T. A. Juniroe, C.A. Scapim and E.S.N. Vieira. 2008. Heterotic Groups in Tropical Maize Gerplasm by Test Cross and Simple sequence Repeat markers. *Genet. Mol. Res.* 7 (4): 1233-1244
7. Cox, T.S., G.L. Lookhart, D.E. Walker, L.G. Harrel, L.D. Albert, and D.M. Rodgers. 1985. Genetic relationship among hard red minter wheat cultivars as evaluated by pedigree analysis and gliadian polyacrilamide gel electrophoretic patterns. *Crop Sci*. 25 : 1058-1063
8. Trindade, A.P.R., R.J.B. Pinto, A.T.D.A. Junior, C.A. Mangolin, M.F.P.S. Machado and C.A. Scapim. 2010. Genetic diversity of breeding popcorn lines determined by SSR markers. Elec. *J. Biothec*. 13: 1-9.
9. Doyle, J.J dan J.L. Doyle. 1987. A Rapid DNA Isolation procedure for small Quantities of Fresh Leaf Tissue. *Phytochemical Bulletin* 19 (1):11-15
10. Kostova. A, E. Todorovska, N. Christov, V. Sevov, and A.I. Atanassov. 2006. Molecular Characterization of Bulgarian Maize Germplasm Collection Via SSR markers. *Biotechnol. & Biotechnol. Eq. Institute of Plant Genetic Resources*, Sadovo, Bulgaria.
11. Krizman, M., J. Jakse, D. Baricewic, B. Javornik dan Prosek. 2006. Robust CTAB-activated Charcoal Protocol for Plant DNA Extraction. *Acta Agriculture Slovenica* 87: 427-433.
12. Wu, Mingsheng, Xihai Jia, L.Tian and Baochun Ly. 2006. Rapid and reliable purity identification of F1 Hybrids of Maize (Zea Mays) using SSR Markers. Moleculer Plant Breeding 4(3): 381-384.
13. Cordeiro, G.M, Y.B. Pan, and R.J. Henry. 2003. Sugarcane microsatellites for the assessment of genetic diversity in sugarcane germplasm. *Plant Science* 165: 181-189.
14. Li, Y.L., D.B.Lv., Y.Z. Wang, S.J. Chen and J.H. Tang. 2004. Study on the genetic diversity of popcorn inbreds and their germpalsm relationship with normal corn inbreds using SSR markers. *Maydica* 49: 327-333.
15. Singh, R.K dan B.D. Chaudhary. 1979. *Biometrical methods in Quantitative genetic Analysis*. Kalyani Publishers. New Delhi.
16. Geographyfieldwork.2011. *Spearman's rank correlation coefficient*. *Barcalona*. *Field Studies Centre*, SL. www. Geographyfieldwork.com.
17. Mosa, H.E. 2010. Estimation of Combining Ability of Maize Inbred Lines using Top Cross Mating Design. *J. Agric. Res*. 36(1): 1-15.
18. Motawel, A. A. 2011. Combining Ability of Some Prolific and Non-prolific Maize Inbred Lines for Grain Yield and Other related Traits. *J. Agric. Res.* El-Sheikh Univ. 37(1): 26-39
19. Sundararajan, R. and P.S. Kumar. 2011. Studies on Combining Ability through Line x Tester Analysis in Maize (Zea Mays. L). *Plant Archiv*. (11)1: 75-77.
20. Rahman, H., I.H. Khalil, N. Islam, Durrishahwar and A. Rafi. 2007. Comparison of original and selected maize populations for grain yield traits. Sardad *J. Agric*. 23: 641-644.
21. Brieger, F.G. 1950. The Basic of Heterosis in Maize. J. Genetic 35:420-427
22. Liu. W and M. Tollenaar. Response of Yield Heterosis to Increasing Plant Density in Maize. *Crop Sci*. 49: 1807-1816.
23. Junior, M.R.C. and W.A. Russell.1988. Evaluation of S_2 Maize Lines Reproduced for Several Generation by Random Mating within Lines.II. Comparison for Testcross Performance of Oiginal and Advanced S_2 and S^8 Lines. *Crop Sci* 2778(29): 899-904.
24. Rafiq, C.M., M. Rafique, A. Hussain and M. Altaf. Studies on Heritability, Correlation and Parth Analysis in Maize (Zea mays L.). *J. Agric. Res*. 48(1): 35-35.
25. Sreckov, Z., A. Nastasic, J. Bocanski, I. Djalovic, M. Vukosavijev and B. Jockovic. 2011. Correlation and Path Analysis of Grain Yield and Morphological Traits in Test-Cross Populations of Maize. *Pak. J. Bot*., 43(3): 1729-1731.
26. Senior M.L, Mutphy J.P., Goodman M.M., Stuber C.W. (1998). Utility of SSRs for determinig genetic similarities and relationships in maize using an agarose gel system. *Crop Sci*. 38: 1088-1098.
27. Srdic, J., S. M. Drinic, Z. Pajic and M. 2007. Pilipovic. Characterisation of maize inbred lines based on molecular markers, heterosis and pedigree data. *Genetika*. 39: 355-363.
28. Mohammadi, S.A., B.M. Prasana, C. Sudan and N.N. Singh. SSR 2008. Heterogeneic pattens of maize parental lines and prediction of hybrid performance. *Faculty of Agronomy, Univ. Of Tabriz. Iran*