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GROUPING IN HETEROTIC POOL OF MAIZE INBRED LINES BASED ON NUMERICAL AND GRAPHICAL ANALYSIS OF COMBINING ABILITY

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SUMMARY

High productivity of cultivars is still the main goal inbreeding programs of many crops in Indonesia. Efforts to increase maize productivity can be undertaken through systematical management of heterosis and combining ability from heterotic pool for establishing new prosperous hybrid cultivars.Hence, the aims of this research were to construct heterotic groups of maize inbred lines and to elucidate the role of the combining ability in the hybrid performance. Hundred hybrids were developed by crossing inbred lines in a complete diallel fashion. The combining ability of inbred lines was evaluated and analyzed by employing Griffing's method 1, cluster analysis and GGE biplot. The result revealed that the general and specific combining abilities could assign inbred lines into three heterotic groups and had a critical role in the hybrid performance for grain yields. Data recorded from reciprocal crosses tended to show better mean and gave more valuable information for hybrid breeding than direct crosses. The use of the combining ability analysis based on Griffing's and GGE biplot has clearly elucidated all characters observed in both general combining ability and specific combining ability. It would provide comprehensive information for genetic analysis in maize breeding. Three heterotic groups were created. The group 1 consisted of three inbred lines, group 2 had only one inbred line and rest were assigned into group 3.

Key words: Maize, diallel, heterotic group, heterotic pool, general combining ability, specific combining ability, reciprocal effect, GGE biplot

Key findings: This research discovered heterotic groups of inbred maize lines and defined good or poor indicators for cross combinations. The importance of the heterotic parameters was noted for comprehensive evaluation of F1s without

neglecting any information. Heterotic groups dissected from numerical and graphical analysis were roughly consistent.

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INTRODUCTION

Maize is well known as an important crop for food and feed materials in nearly every country in the world. In Indonesia, it is ranked the second cereal after paddy. Since the last decade, consumption of this commodity has been increased drastically over national production capacity. This situation causes government to import it around one to three million tons a year. Several efforts have been taken to stimulate the achievement of national production through scheme of extensification and intensification programs. Such programs are generally directed to support national food stock with some additional quantities for industry, and to reduce the risk of imbalance between supply and demand of domestic corn market.

Instead of expanding the production area, high yield cultivar application under an intensification program is a more reasonable alternative to achieve the production target in this country where many agricultural land areas have been abundantly occupied for living houses and industrial use due to the population growth explosion. Hybrid variety is one of the prosperous cultivars, which are explosively adopted in Indonesia due to their promising yield. Breakthrough on a rapid breeding program in hybrid varieties is essential in order to meet the demand on agricultural production. One possible solution is by manipulation of potential genetic pools and appropriate techniques (Arifin *et al.,* 2017). Furthermore, involvement of various genetic attributes is also worth to be considered (Kustanto *et al.,* 2012; Pudjiwati *et al.,* 2013; Rahardjo *et al.,* 2017). Therefore, one practical strategy is heterotic group classification, which separates genetic materials into different pools based on the heterotic behavior of yield characters from their progenies. By such away, the long-term goal of breeding program scan be easily achieved through crossing genetic materials, which are assigned to different groups and not as a speculative way (Badu-apraku *et al*., 2013; Fan *et al*., 2014; Bari *et al*., 2016). The magnitude of the combining ability effect plays a crucial role in heterotic grouping, since it indicates the types of gene action as a preliminary indicator of heterotic expression (Singode *et al*., 2017). A popular method proposed by Griffing (1956) has been widely used for both specific combining ability (SCA) and general combining ability (GCA) from a comprehensive mating design, which is simultaneously employed in the hybrid development (Malik *et al.,* 2004; Zare*et al.,* 2011; Moneam*et al.,* 2015). By simultaneous consideration of the effect of GCA and SCA, several studies have successfully classified maize inbred lines into different heterotic groups, which are presently termed as heterotic group specific and general combining ability(HSGCA) method (Fan *et al*., 2009; Badu-apraku., 2013b). Apart from SCA and GCA, many studies also frequently employ a gene action of the parents derived from the combining ability analysis (Hosana*et al.,* 2015; Ruswandi *et al.,* 2015; Zhang *et al.,* 2016), which becomes an effective way for heterotic parent identification (Rajendran *et al*., 2014). However, as the results of this method are expressed in numerical output, it is difficult to interpret from where their heterotic position is since many crosses would have various combining ability patterns.

The GGE biplot developed by Yan and Hunt (2002) is a graphical method for determining combining abilities, which is widely used for analyzing maize breeding (Bertoia *et al.,* 2006; Khalil *et al.,* 2010; Bocanski *et al.,* 2011; Mostafavi *et al.,* 2012; Ruswandi *et al.,* 2015b). The graphical method is assumed to be more reliable since it can display and predict the combining ability in general patterns by employing first two PCs derived from the principal component analysis (Borghi *et al.,* 2012). In addition, the graphical method can also represent GCA, best mating partners, performance of hybrid combination and heterotic grouping of parental lines in graphic visualization (Bertoia *et al.,* 2006; Dehghani *et al.,* 2012). The simultaneous use of numerical and graphical methods is expected to provide complementary informative results and to enhance interpretation of heterotic groups by means of obtaining a breeding efficiency. This study was aimed to construct heterotic groups in inbred lines and to analyze the role of combining abilities to establish maize hybrid cultivars.

MATERIALS AND METHODS

Experimental design and cultural practices

The research was conducted in two planting seasons from March to November 2017. Development of hybrid combination was performed during the first season, while the evaluation on the combining ability was conducted during the second season. The inbred lines used as parents in this research were developed from polycross mating of Indonesian prosperous commercial varieties. Following that, a recurrent selection up to nine inbreeding generation was conducted. As many as 10 inbred lines, which consisted of *E139, E143, E147, E15, E28, E31, E44, E54, E56* and *Ionby* were used to develop 100 hybrid combinations through the scheme of complete diallel crosses. The hybrid combinations were evaluated with randomized complete block design (RCBD) using 20 m^2 plot units with two replications at the research station of Brawijaya University, Malang, Indonesia

In the first season, all inbred lines were planted side by side in a single row system. Number of 100 seeds were planted with spacing 75 × 30 cm and maintained up to generative stage. Hybridization process was initially performed through flower bagging when the flower shoot, both tassel and silk, has emerged for pollen collection and outcross prevention. Several days later, tassel bag was discarded and the collected pollen was used for pollinating the receptive silk from different inbred lines for making hybrid combinations. The seeds from hybridized plants were harvested in 38 days after pollination, dried up until the seed moisture contents had reached 11%, and further used for hybrid evaluation.

The second step of the research started with seeds preparation. Seeds from each entry (including parents and hybrid combinations) were prepared for as many as 200 seeds. The seeds were further treated with an hour water priming to initiate the germination process. Thereafter, the seeds were dried and stored in plastic bag under moist condition until the seeds started to germinate. After two days, the germinated seeds of each entry were planted in one hole-one seed basis on 75 cm × 15 cm planting space so that there were 150 plants in each plot unit. Appropriate agronomical practices were conducted for supporting the plant growth. Two-time fertilizations were applied with NPK fertilizer with the doses of 60-60-60 kg and 90-90-90 kg on the 10th day and 45th day after planting, respectively. In order to avoid root or stalk lodging, soil hilling was also conducted in the middle of the vegetative stage on 30-35th day after planting. Pesticide and fungicide were periodically sprayed during the vegetative stage with interval of 5 days until the plants reached the generative stage for pest and disease mitigation. Irrigation was provided with interval of 7 days after the emergence of seeds until complete seed setting in the field.

Observations recorded

Observations were recorded at the final stage after ears harvest. The criterion of maturity used for this purpose was when 90% of the husk from the population had dried. All the ears from the standing plants were picked and collected for plot weight scaling. About 15 ears from each entry, which representatively depicted the population were randomly selected for the observation of fresh ear weight (g), shelled ear weight (g) and hundred seed weight (g), whereas the total weight of the ears from each plot was used for grain yield scaling (tha^{-1}). Yield component characters were measured according to the procedure of The International Board for Plant Genetic Resources (IBPGR, 1991), whereas the observation of grain yield was conducted based on the empirical standard suggested by Castellanos-Navarrete *et al.* (2013).

Statistical analyses

Analysis of variance for combining ability based on the Griffing (1956) method 1 with fixed model assumption (model 1) was employed to predict GCA, SCA and the Reciprocal effect. F-test statistical method was used to estimate the significance of GCA, SCA and the reciprocal effect variances, whereas t-test was used to estimate significance of the magnitudes in all effects resulted from the combining ability analysis. Graphical representation of combining ability was drawn based on GGE biplot. Inbred lines as female parents were referred to entries, while the inbred lines as male parents were pointed as testers. Interpretation of SCA and GCA was carried out according to Yan and Hunt (2002) using a polygonal view (which-won-where pattern) and an average tester coordination view, respectively. Graphical analysis was practically done by using *GenStat* v.12.

Heterotic groups were observed by sorting all the numerical data from the observation of yield and yield component characters in decreasing orders. The data were further compared with SCA and GCA of the respective parents with the same arrangement order for a preliminary detection of heterotic parents (Fan *et al.,* 2013). The existence of the highest mean accompanied with the highest combining ability was the basic consideration for the heterotic classification. A conclusion of heterotic groups was made by subjecting the SCA and GCA data for a cluster analysis. An unweighted pair group method with arithmetic averages (UPGMA) based on dissimilarity coefficient of Euclidean was employed for making heterotic group summary of numerical data into a cluster graph (Rajendran *et al.,* 2014), whereas graphical representation of heterotic group was determined by the ordinate position of entries in the average tester coordination view of GGE Biplot. In order to ensure the heterotic patterns among inbred lines in cross combination, mid parent heterosis (MPH) and high parent heterosis (HPH) were also performed with the formulation as suggested by Hallaueret *al.* (2010).

$$\mathrm{MPH} = \frac{\mathrm{F1{-}MP}}{\mathrm{MP}} \text{ x } 100\%$$

$$\mathrm{MPH} = \frac{\mathrm{F1{-}HP}}{\mathrm{HP}} \text{ x } 100\%$$

Where F1 is hybrid mean, MP is average performance from male and female parents of the respective hybrid, and HP is mean of high parents in each hybrid combination. Pearson correlation was further employed to predict relationship between heterotic parameters with grain yield performance.

RESULTS

Analysis of variance combining ability

The results of analysis of variance (ANOVA) for combining ability were written in Table 1. The mean squares of genotype in all characters revealed significant differences ($P < 0.05$). Thus, partitioning genotype sources of variance into GCA, SCA and reciprocal effects was possible. All the mean squares of both GCA and SCA exhibited similar pattern, which had highly significant difference in all characters. Characters of shelled ear weight were the only characters, which were found to have a significant value in the reciprocal effect and make them considered in further evaluation. The relative importance of the combining ability by the ratio of GCA:SCA revealed a value of more than 1 in all characters. It was indicated that additive type of a gene action gave a higher contribution to genetic variation than non-additive type of the gene action.

General combining ability

General combining abilities (GCA) estimated from inbred lines were given in Table 2.The details of the GCA analysis revealed that inbred *E139, E31* and *E44* had a significant positive GCA for the fresh ear weight, while inbred *E139, E147, E15, E31, E44, E147* and *E15* had a significant positive GCA for the shelled ear weight. The best GCA for hundred seed weight was inbred E15 and E28, while inbred E15 was the only inbred which had a positive significant GCA for the grain yield. Desirable GCA effects of such inbred lines might be useful for further economic trait

Table 1. Mean square of combining analysis in yield components and grain yield.

Source	DF	FEW	SEW	HSW	GY
Replication	1	249.20	3.54	3.83	1.78
Genotypes	99	3410.04**	2634.87**	52.19**	4.30**
GCA	9	3418.30**	3066.49**	58.84**	4.32**
SCA	45	2754.01**	2001.69**	40.58**	3.05**
REC.	45	325.23	255.56*	5.06	0.81
Error	99	263.13	161.26	3.58	0.72
GCA:SCA		1.24	1.53	1.45	1.42

*,** significant at 0.05 and 0.01 probability levels for a t-test, respectively, GCA: General combining ability, SCA: Specific combining ability, REC: Reciprocal effect, DF: Degree of freedom, FEW: Fresh ear weight (g), SEW: Shelled ear weight (g), HSW: Hundred seed weight (g) and GY: Grain yield (t ha^{-1}).

Table 2. General combining ability (GCA) of ten inbred lines in yield components and grain yield.

Inbreds	FEW	SEW	HSW	GY
E139	6.91*	9.22**	-0.63	0.14
E143	-26.59**	-21.13**	-2.59**	-0.66**
E147	6.12	7.38**	0.57	0.25
E15	2.31	5.81*	2.97**	0.84**
E28	-1.81	2.28	1.75**	0.35
E31	16.19**	13.72**	0.52	0.02
E44	16.86**	13.54**	0.73	-0.14
E54	-0.60	-4.09	0.02	0.06
E56	-10.78**	-15.53**	-2.31**	-0.75**
Ionby	-8.62*	-11.21**	-1.01*	-0.11
SE	3.44	2.69	0.40	0.18
CD-0.05	6.83	5.35	0.80	0.36
CD-0.01	9.04	7.08	1.06	0.47

*,** significant at 0.05 and 0.01 probability levels for a t-test, respectively, SE: Standard error, CD-0.05: Critical differences at 0.05 probability level, CD-0.01: Critical differences at 0.01 probability level, FEW: Fresh ear weight (g), SEW: Shelled ear weight (g), HSW: Hundred seed weight (g) and GY: Grain yield (t ha^{-1}).

improvement. The comprehensive observation of GCA revealed that two categories, both positive and negative, were successfully identified in all inbred lines. All the yield component characters revealed that inbred lines: *E139, E147, E15, E28, E31* and *E44* tended to have GCA in a positive direction, whereas the others *viz*. *E143, E54, E56* and *Ionby* had a negative GCA. Similarly, the GCA effect on the grain yield revealed that similar inbred as classified in yield components also had positive and negative values, respectively.

Performance and specific combining ability of direct crosses

The mean values and SCA effects of 45 direct crosses for four characters were illustrated in Table 3. SCA depicted the relative value of specific cross towards grand mean of all cross combinations in certain characters. This leads to the fact that the rank of SCA will not always conform with the mean performance of a inbred cross. Consideration in both mean and SCA is valuable for selection or summarization of the genetic potential of inbred lines. Based on the data given, the patterns of hybrids having

Table 3. Mean performance and specific combining ability (SCA) of direct crosses.

Hybrids	FEW	SCA	SEW	SCA	HSW	SCA	GY	SCA
E139 × E143	203.44	12.45	160.84	10.42	37.46	1.94	8.89	0.22
E139 × E147	191.11	-12.40	152.06	-10.72	36.58	0.26	8.35	-0.02
E139 × E15	188.00	4.08	154.06	5.39	35.54	-2.18	8.18	-0.13
E139 × E28	215.60	5.76	170.23	0.10	37.33	-0.91	8.24	-0.42
E139 × E31	207.50	-2.56	165.65	-2.95	36.83	-0.23	7.18	-0.67
E139 × E44	196.83	1.72	156.95	3.12	34.92	-0.94	7.36	0.18
E139 × E54	232.04	34.07**	182.05	29.96**	40.25	4.39**	9.36	1.29*
E139 × E56	223.67	19.55	166.41	15.89	37.83	3.76**	8.16	-0.15
E139 × Ionby	212.02	34.58**	165.87	32.01**	36.46	3.61**	7.46	0.56
E143 × E147	190.79	27.77**	146.41	24.04**	38.08	2.06	6.94	0.73
E143 × E15	214.46	31.55**	169.08	26.98**	40.88	3.08*	9.15	0.72
E143 × E28	190.88	27.19*	150.67	23.46**	39.58	2.67*	8.69	1.45**
E143 × E31	211.23	11.51	167.93	12.81	37.92	2.65*	8.06	0.59
E143 × E44	190.80	16.19	144.88	13.97	36.63	2.24	6.93	0.78
E143 × E54	114.80	-13.20	71.58	-3.23	26.37	-2.25	5.86	-0.10
E143 × E56	152.02	-15.32	81.32	-29.85**	23.13	-7.41**	4.00	-2.17**
E143 × Ionby	124.63	-34.56**	76.97	-33.09**	25.03	-5.23**	5.08	-1.67**
E147 × E15	199.62	2.56	161.64	-4.36	40.33	0.67	8.33	-1.02
E147 × E28	174.75	-7.87	144.40	-7.43	38.42	-0.25	8.30	-0.58
E147 × E31	197.76	2.79	158.04	-4.68	37.54	-2.76*	7.86	-0.25
E147 × E44	214.44	5.79	168.44	8.45	35.42	-0.62	8.51	0.73
E147 × E54	191.60	6.38	150.95	9.62	39.33	3.55**	7.52	-0.07
E147 × E56	195.46	15.52	154.98	15.27	37.25	1.78	8.49	0.80
E147 × Ionby	228.50	37.49**	176.67	32.25**	40.17	3.38**	7.67	0.68
E15 × E28	171.67	-11.57	132.46	-15.25	39.04	-2.12	7.45	-1.34*
E15 × E31	207.93	-3.88	162.44	-4.04	39.46	-3.07*	8.64	-0.43
E15 × E44	206.36	-0.02	155.64	-3.13	39.21	-1.53	7.21	-0.67
E15 × E54	212.83	28.77**	170.17	30.79**	45.96	5.88**	9.79	1.91**
E15 × E56	182.71	14.10	137.42	13.05	41.83	2.96*	8.81	1.42*
E15 × Ionby	204.71	22.57*	165.67	26.56**	44.00	5.29**	9.31	1.81**
E28 × E31	211.56	-6.73	160.38	-5.98	36.75	-2.86*	8.09	-0.27
E28 × E44	222.09	-0.96	175.79	-1.72	39.04	-2.30	7.60	-0.80
E28 × E54	231.46	34.85**	184.92	30.78**	45.58	6.12**	11.18	2.64**
E28 × E56	194.08	15.95	155.25	20.68*	40.75	3.43**	7.44	0.03
E28 × Ionby	213.63	33.81**	171.38	33.48**	43.88	6.52**	10.32	2.06**
E31 × E44	269.86	37.07**	196.96	19.39*	37.92	-0.14	8.82	0.74
E31 × E54	207.24	7.49	150.60	6.56	39.92	3.54**	7.52	-0.28
E31 × E56	212.15	39.25**	161.18	34.32**	40.13	4.16**	7.86	1.47**
E31 × Ionby	201.05	21.89*	159.55	26.22**	37.38	3.53**	8.19	0.74
E44 × E54	232.31	19.45	175.58	14.74	38.08	1.23	7.44	-0.50
E44 × E56	213.96	18.60	164.62	20.15*	40.25	3.31**	8.67	1.15*
E44 × Ionby	228.38	33.18**	176.97	27.31**	41.58	5.43**	8.29	0.88
E54 × E56	144.87	-29.66**	103.96	-26.55**	29.20	-5.92**	6.81	-1.16*
E54 × Ionby	178.84	-16.44	85.10	-43.13**	24.87	-9.37**	5.55	-1.89**
E56 × Ionby	132.04	-31.58**	73.88	-34.88**	24.23	-6.89**	5.13	-1.51**
SE		10.39		8.13		1.21		0.54
CD-0.05		20.61		16.13		2.41		1.08
CD-0.01		27.28		21.36		3.18		1.43

*,** significant at 0.05 and 0.01 probability levels for a t-test, respectively, SE: Standard error, CD-0.05: Critical differences at 0.05 probability level, CD-0.01: Critical differences at 0.01 probability level, FEW: Fresh ear weight (g), SEW: Shelled ear weight (g), HSW: Hundred seed weight (g) and GY: Grain yield (t ha^{-1}).

the highest mean and desirable SCA in all characters studied were detected from the 6 inbred crosses (*E31, E44, E139, E28, E147*) × three inbreeds (*E54, Ionby and E56*). Inbred *E143* tended to be inferior with low to moderate observation values of mean and SCA effect although some cases also revealed desirable performance. In decreasing order, the cross of (*E31* × *E44*) was detected to have the best mean and roughly had a positive significant SCA effect for fresh ear weight and shelled ear weight followed by the cross of (*E44* × *E54*), (*E139* × *E54*), (*E28* × *E54*), (*E147* × *Ionby*) and (*E44* × *Ionby*). Thirteen hybrid combinations revealed the highest mean above 40 g and 8 t ha^{-1} for hundred seed weight and grain yield respectively. The most considerable crosses were in (*E15* × *E54*), (*E28* × *E54*), (*E15* × *Ionby*), (*E28* × *Ionby*), (*E15* × *E56*), (*E139* × *E54*) and (*E44* × *E56*), since they had both the highest mean and SCA in the hundred seed weight and grain yield characters.

Performance and reciprocal effect of reciprocal crosses

Table 4 revealed the mean and reciprocal effects from the reciprocal mating pairs from inbred lines. Mathematical formulation for reciprocal effects was assumed as the half of the gap between direct crosses and their reciprocal. Thus, well performed reciprocal crosses tended to have negative effects and can be further considered for a desirable parameter. Reciprocal crosses for fresh ear weight and shelled ear weight revealed that ten inbred crosses were well performed. Among the selected crosses, *E56* × *E31* was identified as the most superior for ear part since it had the most desirable reciprocal effect in addition to the highest mean in fresh ear weight and shelled ear weight, respectively. The crosses of (*E44* × *E31*), (*Ionby* × *E31*), (*E44* × *E139*), (*Ionby* × *E139*), (*E54* × *E139*) and (*E54* × *E15*) can also be considered to have an expected performance although they did not have a significant effect in negative direction. In the characters of hundred seed weight and grain yield, there were four prospective inbred crosses, which had considerable performance and reciprocal effects, including (*E54* × *E15*), (*Ionby* × *E28*), (*Ionby* × *E15*) and (*E54* × *E28*). Interestingly, the most negative significant reciprocal effect was not detected to have the best mean performance in such characters.

Graphical analysis of combining ability

The graphical analysis of the combining abilities from the principal component scores was drawn in Figure 1a-d for GCA as the average tester coordinate (ATC) biplot. The magnitude of GCA was assessed according to entry position on ATC abscissa (the line with one arrow). The variations accounted by each biplot were 62.48%, 68.66%, 82.13% and 76.24% for the fresh ear weight, shelled ear weight, hundred seed weight and grain yield, respectively. Because the average variation was greater than half of the total variation, it could be noted that graphical representation was predictive for all characters. The biplot indicated that the magnitudes of GCA in the decreasing order of fresh ear weight (Figure 1a) were in following: *E44, E139, E28, E147, E15* (positive*), E54, E56, E143* and *Ionby* (negative). In

Table 4. Mean performance and reciprocal effect (REC.) of reciprocal crosses.

Hybrids	FEW	REC.	SEW	REC.	HSW	REC.	GY	REC.
E143 × E139	167.68	17.88	130.90	14.97	34.17	1.65	6.44	1.22*
E147 × E139	195.72	-2.31	154.40	-1.17	38.00	-0.71	8.33	0.01
E15 × E139	224.19	-18.09	181.50	-13.72	38.96	-1.71	9.46	-0.64
E28 × E139	191.70	11.95	147.68	11.28	37.29	0.02	7.84	0.20
E31 × E139	219.16	-5.83	169.04	-1.70	36.67	0.08	7.74	-0.28
E44 × E139	239.74	-21.46	189.51	-16.28	37.58	-1.33	8.93	-0.79
E54 × E139	234.31	-1.14	182.85	-0.40	41.50	-0.63	9.57	-0.10
E56 × E139	193.26	15.21	147.47	9.47	38.00	-0.08	6.26	0.95
Ionby × E139	239.30	-13.64	188.88	-11.51	41.67	-2.60	9.67	-1.11
E147 × E143	209.37	-9.29	168.89	-11.24	36.17	0.96	9.63	-1.35*
E15 × E143	185.67	14.40	148.96	10.06	40.21	0.33	8.58	0.28
E28 × E143	192.29	-0.71	153.29	-1.31	38.25	0.67	9.52	-0.42
E31 × E143	176.57	17.33	137.60	15.17	37.42	0.25	7.76	0.15
E44 × E143	207.70	-8.45	162.60	-8.86	38.29	-0.83	8.96	-1.02
E54 × E143	190.00	-37.60**	176.20	-47.33**	38.15	-5.89**	8.68	-1.41*
E56 × E143	128.17	11.93	80.38	0.47	26.41	-1.64	4.77	-0.38
Ionby × E143	121.40	1.61	86.89	-4.96	31.47	-3.22*	5.97	-0.45
E15 × E147	207.93	-4.15	150.73	5.45	42.25	-0.96	7.74	0.29
E28 × E147	203.70	-14.48	154.79	-5.20	39.92	-0.75	7.68	0.31
E31 × E147	238.00	-20.12	169.51	-5.74	33.29	2.13	8.13	-0.14
E44 × E147	228.69	-7.13	185.00	-8.28	40.13	-2.35	9.10	-0.29
E54 × E147	217.77	-13.09	169.58	-9.31	43.13	-1.90	8.89	-0.69
E56 × E147	211.83	-8.19	153.96	0.51	37.00	0.13	8.06	0.22
Ionby × E147	227.04	0.73	174.89	0.89	39.88	0.15	9.91	-1.12
E28 × E15	191.79	-10.06	147.96	-7.75	40.33	-0.65	8.18	-0.36
E31 × E15	206.89	0.52	163.26	-0.41	35.54	1.96	8.15	0.25
E44 × E15	217.53	-5.58	171.51	-7.93	39.29	-0.04	8.79	-0.79
E54 × E15	233.71	-10.44	189.58	-9.71	45.96	0.00	11.76	-0.99
E56 × E15	214.13	-15.71	163.96	-13.27	39.58	1.13	10.15	-0.67
Ionby × E15	213.38	-4.33	171.38	-2.85	44.67	-0.33	11.71	-1.20*
E31 × E28	189.32	11.12	154.40	2.99	36.25	0.25	8.06	0.01
E44 × E28	191.69	15.20	147.13	14.33	35.50	1.77	7.15	0.23
E54 × E28	219.00	6.23	167.75	8.58	44.38	0.60	10.86	0.16
E56 × E28	198.23	-2.07	154.33	0.46	39.17	0.79	7.75	-0.16
Ionby × E28	218.71	-2.54	172.46	-0.54	44.83	-0.48	10.23	0.04
E44 × E31	255.96	6.95	191.06	2.95	38.46	-0.27	8.35	0.23
E54 × E31	224.49	-8.62	176.50	-12.95	42.42	-1.25	8.04	-0.26
E56 × E31	262.75	-25.30*	198.56	-18.69*	38.79	0.67	9.56	-0.85
Ionby × E31	243.43	-21.19	192.64	-16.54	42.88	-2.75*	9.05	-0.43
E54 × E44	224.69	3.81	167.52	4.03	40.04	-0.98	7.35	0.05
E56 × E44	220.97	-3.50	166.40	-0.89	37.38	1.44	7.80	0.44
Ionby × E44	240.04	-5.83	177.02	-0.02	42.88	-0.65	8.91	-0.31
E56 × E54	158.63	-6.88	98.41	2.78	28.55	0.33	5.43	0.69
Ionby × E54	155.41	11.71	92.75	-3.83	28.59	-1.86	6.51	-0.48
Ionby × E56	151.56	-9.76	97.61	-11.87	29.52	-2.64	6.06	-0.46
SE		11.47		8.98		1.34		0.60
CD-0.05		22.76		17.82		2.66		1.19
CD-0.01		30.13		23.58		3.52		1.57

*,** significant at 0.05 and 0.01 probability levels for a t-test, respectively, SE: Standard error, CD-0.05: Critical differences at 0.05 probability level, CD-0.01: Critical differences at 0.01probability level, FEW: Fresh ear weight (g), SEW: Shelled ear weight (g), HSW: Hundred seed weight (g) and GY: Grain yield (t ha^{-1}).

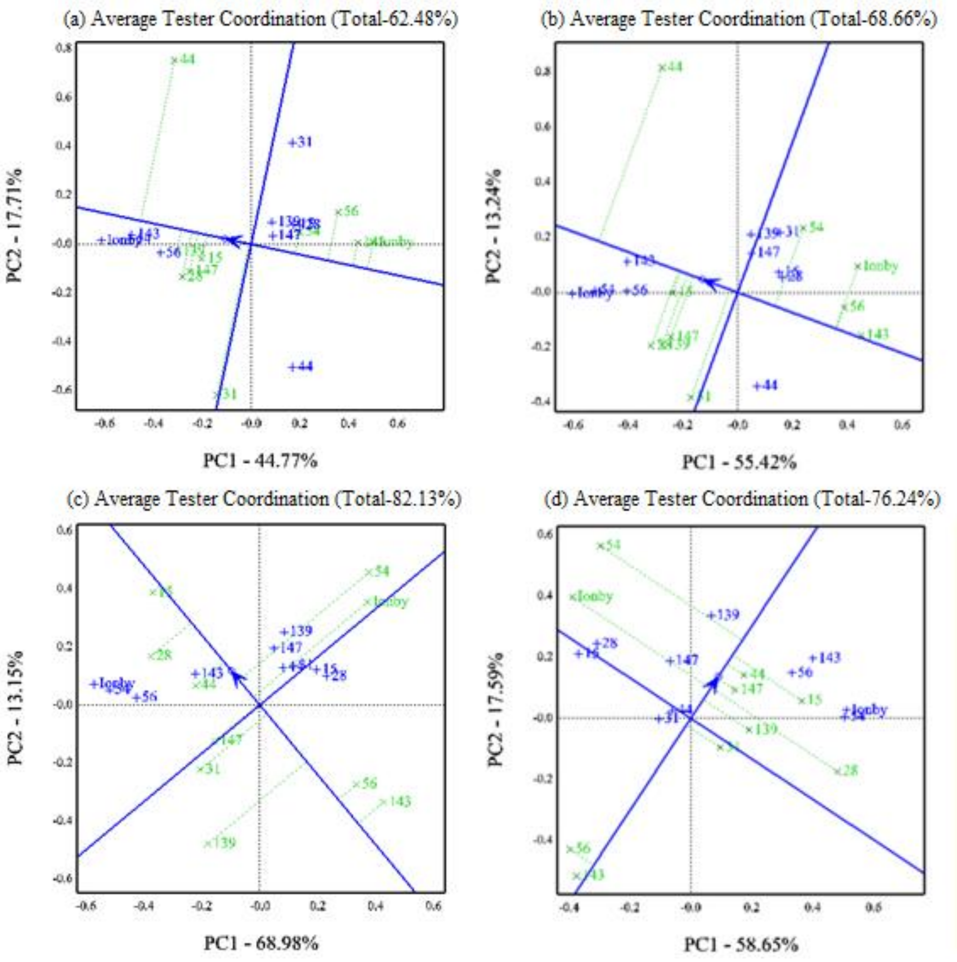

Figure1 (a-d). Average tester coordination for fresh ear weight (a), Shelled ear weight (b), hundred seeds weight (c) and grain yield (d). Inbred lines are labeled with green color when viewed as entry and labeled with blue color when viewed as tester.

shelled ear weight, it was detected that the average tester coordinate marker revealed five entries, which had a positive GCA effect in the order of *E44, E28 E15, E139* and *E147*, whereas the entries *E54, E56 Ionby* and *E143* were identified to have a negative GCA effect (Figure 1b). The GCAs of entries for hundred seed weight were approximated in Figure 1c, which displays information of six entries *viz. E15, E28, E44, E54, Ionby* and *E147* having a positive GCA and *E31, E139, E56* and *E143* having a negative direction. The average tester coordination view of entries in Figure 1d demonstrates the GCA for the grain yield. The biplot showed that all entries tended to have a positive GCA. The positive GCA was showed by seven entries with the order of *E54, E15, E44, E147, E28 Ionby* and *E139*, whilst entry *E31, E56* and *E143* were known to have a negative GCA.

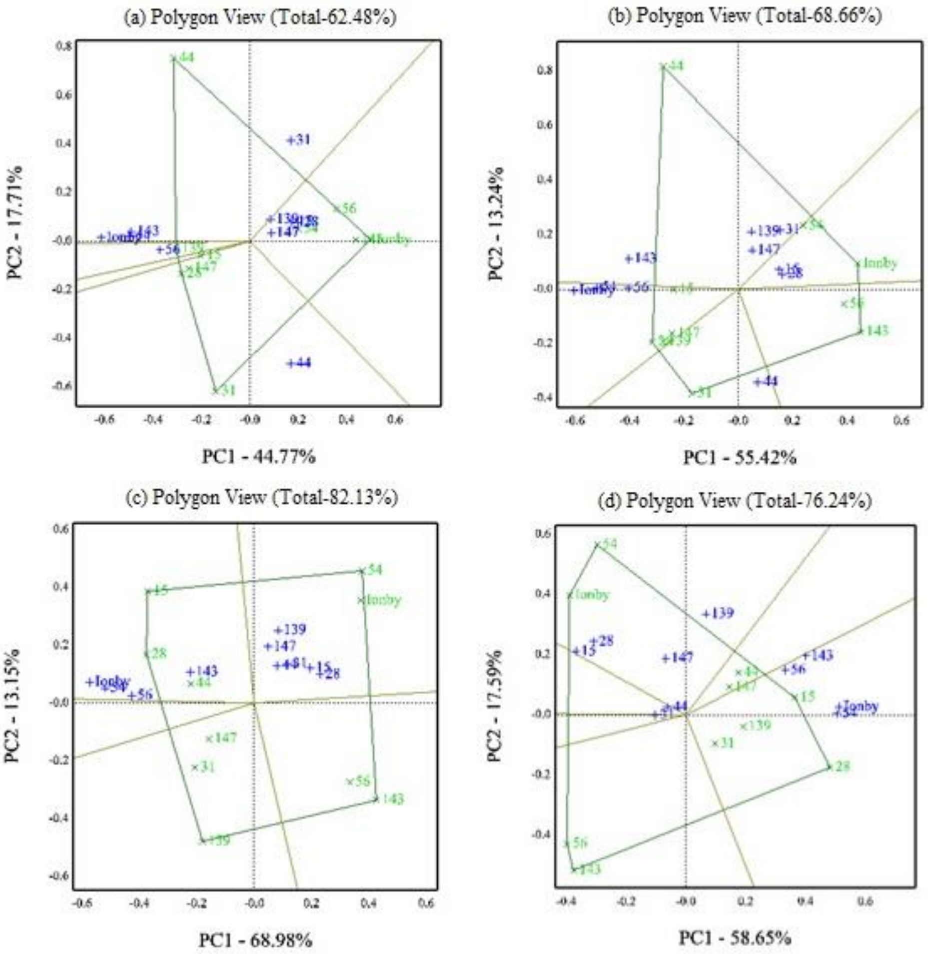

Figure2 (a-d). Polygon view for fresh ear weight (a), Shelled ear weight (b), hundred seeds weight (c) and grain yield (d). Inbred lines are labeled with green color when viewed as entry and labeled with blue color when viewed as tester.

Figure 2a-d was the polygon view as representation of SCA of the crosses among inbred lines. The polygon divides in several sectors, by which converge of entry and tester in respective sector indicate that cross combinations confer desirable SCA. The polygon view of the fresh ear weight (Figure 2a) revealed that entry *E54, E56, E143* and *Ionby* had the best SCA in the mating pairs with tester *E15, E28, E15 and E147*. Entry *E15, E147, E28* and *E31* had the highest SCA as mated with tester *E44*, whilst tester *E31, E56, E143, E54* and *Ionby* shared the best SCA with entry *E139* and *E44*.

In shelled ear weight characters (Figure 2b), entry *E54* and *Ionby* were predicted as the best mating partner for tester *E15* and *E28*. Entry *E31* was predicted to have a specific mating partner with tester E44. In other crosses, entry *E28, E139, E147* and *E15* revealed best SCAs in the hybrid combination with *Ionby*, *E54* and *E56* as testers, while combinations of entry *E44* with tester *E143, E139, E147* and *E31* also showed desirable SCAs.

The most desirable SCA of hundred seed weight was only depicted by two sectors from the biplot (Figure 2c). Information summarized in the first sectors is that entry E54 and *Ionby* produced better performance and SCA in mating pairs with *E139, E147, E44, E31, E15* and *E28*. Second sector informs that entry *E15, E28* and *E44* shared the best SCA in mating pairs with tester E143*, E56, E54 and Ionby.* Figure 2d describes the SCAs for the grain yield. Graphical visualization reveals that entry *E31, E139, E15* and *E28* had the best SCAs in mating combinations with tester *E56, E143, E54* and *Ionby*. Moreover, two entries *viz*. *Ionby* and *E54* also revealed considerable SCAs as mated with tester *E147, E139, E15* and *E28.*

Expression of heterosis in grain yield

Heterosis is useful for elucidating the genetic distinctness of inbred lines. Therefore, expression of this parameter should be comprehensively considered with the other parameters for dissecting the heterotic group of maize inbred lines. Performances of mid parent and high parent heterosis for grain yield are illustrated in Figure 3. The figure showed that almost all hybrids exhibited positive mid parent and high parent heterosis. Out of ninety hybrids, it was found that most hybrids derived from the crosses of line *E139, E147, E15, E28, E31* and *E44* tended to express powerful gain as mated with line *E143, E54, E56* and *Ionby* both as male and female parents. It was roughly depicted by the crosses of (*E143* × *E28*), (*E143* × *E31*), (*E147* × *E143*), (*E15* × *Ionby*), (*E28* × *E143*), (*E28* × *E54*), (*E28* × *Ionby*), (*E44* × *E143*) and (*E54* × *E15*). Conversely, poor heterosis values were exhibited by the most hybrid combinations from the crosses within the inbred, which are classified above as revealed by the crosses of (*E139* × *E31*), (*E15* × *E47*), (*E139* × *E28*), (*E15* × *E28*), (*E56* × *E143*) and (*E143* × *E56*). Inbred *Ionby* seemed to have a different heterosis pattern with others since it exhibited stable heterotic expression and higher value in cross combinations.

Heterotic group

Preliminary detection of heterotic groups was tested by the pattern of mean values in yield components and grain yield in decreasing order and their SCAs and GCAs. The highest mean in yield components and grain yield showed that several inbred crosses had considerable mean accompanied with positive significant SCAs and positive significant GCAs in one of the parents. This phenomenon indicates that different heterotic groups exist among inbred lines. The summary of heterotic groups based on the SCAs and GCAs in 10 inbred lines using cluster analysis is presented in Figure 4. The possibility of 3 heterotic groups from all inbred lines as the sources of desirable genetic pools is clearly elucidated based on the numerical data. Group 1 consisted of 3 inbred lines (*E54, E143 and E56*), Group 2 consisted of 1 inbred lines (*Ionby*) and 6 others (*E28, E139, E15, E147, E44* and *E31*) were assigned in Group 3. The results from the cluster analysis are also supported by the value of heterosis, by which cross combinations among inbred lines within each group tended to have lower mid and high parent heterosis than cross combinations among groups.

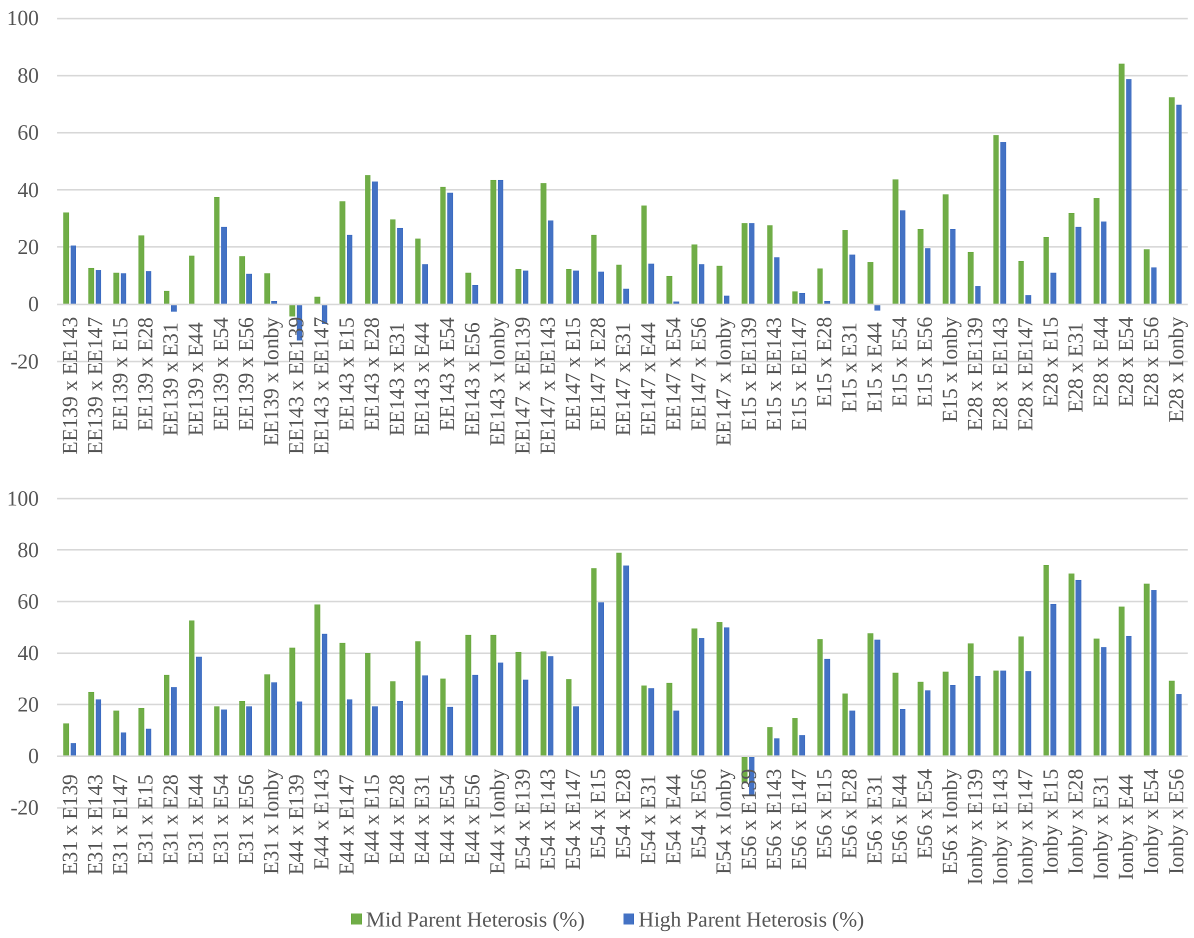

Figure 3. Heterosis of grain yield in F1.

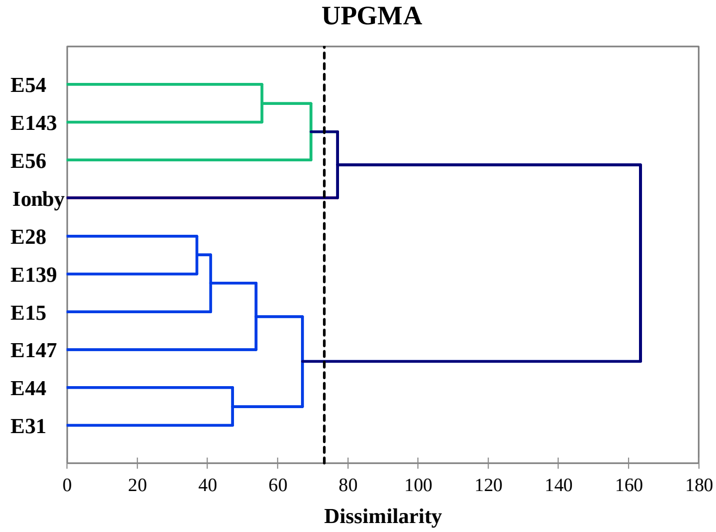

Figure 4. Heterotic group of 10 inbred lines based onthe cluster analysis

Heterotic grouping based on the ordinate position of the average tester coordination view (line without arrow) in GGE biplot also revealed similar heterotic patterns among inbred lines in cross combinations (Figure 1a-d). However, inbred Ionby tended to be grouped with inbred *E54, E143* and *E56,* while inbred *E28, E139, E15, E147, E44* and *E31* were clearly assigned in the same group as shown in the numerical method.

Relationship between grain yield and heterotic parameters

The results of Pearson correlation between grain yield and heterotic parameters are listed in Table 5. All heterotic parameters including SCAs, GCAs in male and female parents in cross combination, and mid and high parent heterosis were positively correlated and significant to the grain yield. Only one parameter namely reciprocal effect was significantly correlated to the grain yield performance in the negative direction. Among heterotic parameters, heterosis in both mid and high parents had negative significant correlation with the reciprocal effects. However, they were detected to have positive significant correlation with SCA effects. No relationship was observed between SCA and GCA to the reciprocal effects. Thus, it indicates that such parameters are independent.

DISCUSSION

The understanding of combining ability is profoundly substantial in plant breeding since it has an important role to predict the portion of gene actions, which govern the trait expression. The GCAs and SCAs from the partitioned sources of genotype variation reveal highly significant effects in all characters, pronouncing that additive and non-additive types of gene actions work together to build genetic variation in cross combinations. Explanation of this phenomena is more completed because the predicted value from the ratio between GCA:SCA mean squares has a greater value than one in all characters studied. It implies that additive types of gene actions are dominantly expressed and inherited by the parents to their progeny while non-additive types of gene action had a complementary effect in plant phenotype. This result is in agreement with the finding of Amegbor *et al.* (2017) who found a similar result across environment trial.

As depicted by the significance of the GCA mean square, a number of

Table 5. Pearson correlation among heterotic parameters.

	GY	REC	SCA	GCA fp	GCA mp	MPH
REC	-0.45*					
SCA	0.79*	0.00				
GCA fp	0.33*	-0.02	0.06			
GCA mp	0.31*	0.02	0.06	-0.11		
MPH	0.57*	-0.44*	0.49*	-0.02	0.03	
HPH	0.48*	-0.40*	0.43*	-0.05	-0.01	0.96*

*Significant at 0.05 level, GY: grain yield (t ha^{-1}), REC: Reciprocal effect, SCA: Specific combining ability, GCA fp: GCA of female parents in crosses combination, GCA mp: GCA of male parent in crosses combination, MPH: Mid parent heterosis and HPH: High parent heterosis.

six inbred lines namely, *E139, E147, E15, E28, E44* and *E31* possess the most desirable GCAs in almost all characters. These lines may be considered for use in specific crosses to build superior characters because they accumulate gene blocks or chromosome segments that additively act for character improvement (Singode *et al*., 2017). Similarly, significance of SCAs and reciprocal mean square are also accompanied by many cross combinations, which have significant SCA effects giving a clue that allelic diversity among inbred lines exists and directly corresponds thereby indicating the presence of distinct genetic pools (Kanyamasoro*et al.,* 2012; Nepir*et al.,* 2015; Fan *et al.,* 2008; Fan *et al.,* 2013; Bari *et al.,* 2016) with different portion of maternal effects (Rajendran *et al*., 2014; Zhang *et al*., 2016).

The common method in heterotic group classification from phenotypic characteristic is based on SCAs (Kanyamasoro *et al.,* 2012), combination in both SCAs and expression of important characters (Noelle *et al*., 2017) and GCAs and SCAs of multiple traits (Fan *et al*., 2009; Badu-Apraku *et al*., 2013; Badu-Apraku *et al*., 2013b; Fan *et al*., 2013). In this research, efforts to classify heterotic groups as an indicator of the presence of different genetic pools have been done and successfully identified 3 heterotic groups from 10 inbred lines based on the cluster analysis of numerical data (GCA and SCA). Inbred *E143, E54* and *E56* are classified in group 1, inbred *Ionby* is classified in group 2 while inbred *E139, E147, E15, E28, E31* and *E44* are classified in group 3. Several facts and features from the heterotic groups elucidated from this research can also describe the behavior of combining ability and character expression as previously reported by many studies. The inbred lines assigned in group 1 and 2 have negative GCA effects, whereas inbred lines assigned in group3 tend to have positive GCA effects. It was unintentionally revealed that additive types of the gene action as indicated by inbred's GCAs have a potential as a determinant factor in heterotic grouping as reported by Fan *et al*. (2008) and Nepir *et al*. (2015). Moreover, attractive and poor performances exist among inbred lines in intergroup and intra group crosses, respectively. It indicates that inbred lines from respective groups have divergent allele contents in each locus and can build a complex allelic complementation and *vice versa* (Sugiharto *et al*., 2018). The same finding was also reported by Bidhendi *et al*. (2012) in grouping maize lines using diallel crosses.

In the perspective of graphical analysis through GGE biplot, only 2 groups were visualized. Inbred *Ionby* that was previously assigned to a distinct group by the numerical method, was surprisingly assigned in the same group together with *E143, E54* and *E56*, whereas others remained in respective groups. It was also attributed by different ranks of inbred lines and cross combinations for GCA, SCA and reciprocal effects derived from both methods. The similar phenomenon was also found by several researchers in studying numerical and graphical analyses of combining abilities accompanied with heterotic grouping (Khalil *et al*., 2010; Bocanski *et al*., 2011). Speculation about this phenomenon occurs due to the different amount of variation accounted by each method. GGE biplot accounts for 62.48%, 68.66%, 82.15% and 76.24% for the fresh ear weight, shelled ear weight, hundred seed weight and grain yield, respectively, whereas the numerical method consider total variation from all cross combinations. The remaining uncounted variation might be the main cause that reflects the results detected by both methods. Furthermore, heterotic parameters calculated by the numerical method such as GCAs and SCAs are based on the average value of inbred lines as males or females, whilst the graphical method separately uses both males and females in prediction. The use of both methods in a simultaneous way might be more valuable from test cross, which separately uses inbred lines as male and female parents like Line × Tester analysis as reported by Ruswandi *et al.* (2015b), which shows a high conformity level. The conformity of the numerical method from Griffing (1956) and graphical methods suggested by Yan and Hunt (2002) are still unclear since the results from the analysis are not completely conserved across the cross combinations. However, the general results from the numerical analysis in this study are consistent with those of the graphical analysis. It is similar to the finding of most researchers in related work (Borghi *et al*., 2012; Rastogi *et al*., 2013).

The attributes of heterotic group as indicated by the magnitude of heterotic parameters denote that these parameters have a critical role in genetic improvement. The best cross combinations revealing desirable yield components and grain yield performance tend to have female parents or one parent with good GCAs (both significant positive and positive), whereas the undesirable cross performance has male and female parents with poor GCAs. This result indicates that the cross combinations with the pattern of additive × non-additive type of the gene action are profoundly important and can be subjected as basic consideration for across breeding program. The similar result was also found by Matin*et al.* (2016) and Assuncao *et al.* (2010), who noted that cross combinations by incriminating one parent with high portion of additive effect can manifest good performance. Pearson correlation has also been done and revealed that not only GCA, but also SCA, reciprocal effects and heterosis (MPH and HPH) have significant effect to the grain yield. Inheritance of dominant and over dominant effects from complementary role of a non-additive gene action in the parents may also be supported by cytoplasmic genes in the expression of the grain yield. The most significant heterotic parameter was SCA. The work conducted by Badu-Apraku *et al.* (2013) and Singh *et al.* (2015) in maize and Zhang *et al*. (2015) in barley also support this finding.

CONCLUSION

The results of this research used the magnitude of GCA and SCA as a parameter of heterotic group classification without excluding any superior hybrid of yield components and grain yield in three distinct groups. Inbred *E143, E54* and *E56* were assigned in the group 1, Inbred *Ionby* was in the group 2, and inbred *E139, E147, E15, E28, E31* and *E44* were placed in the group 3. The role of parent’s GCA in the hybrid is essential, by which desirable direction of GCA in one parent can complement other parents with poor GCA forming additive × non-additive interaction. In general, the results of numerical and graphical methods for the combining ability analysis are roughly consistent. A few numbers of dissimilarities can be caused by the different calculation foundation of both methods in the combining ability and heterotic parameters. Heterotic parameters are complexly contributed to the grain yield performance and can simultaneously be assessed in breeding programs.

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