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Original Article

Comparison of hybrid vigor based on parental distance in SSR markers and agronomic traits in mungbean (*Vigna radiata* (L.) Wilczek)

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Abstract

Heterosis of seed yield in mungbean (*Vigna radiata* (L.) Wilczek) has created an interest among plant breeders to develop hybrid mungbean cultivars. The objective of this study was to compare levels of heterosis among four F_1 hybrids of mungbeans with different genetic distance. The hybrids were developed by using Sukhothai (SKT) as the female parent and pollinated by male parents of different genetic distance as revealed by SSR markers. They were H192 (close distance), C357 (moderate distance), TC1965 (high distance) and W166 (very high distance). The results revealed that the F_1 from the parents with larger genetic distance showed higher heterosis in yield per plant and number of pods per plant. Thus SSR markers combined with yield components can be used to identify parental lines with high genetic distance for hybrid seed production in mungbean. This approach potentially helps to reduce the amount of fieldwork required for evaluation of F_1 hybrids.

Keywords: heterosis, genetic distance, parent diversity, simple sequence repeat markers, yield component

1. Introduction

Mungbean [*Vigna radiata* (L.) Wilczek: Leguminosae] is a short-lived annual legume crop cultivated mainly in Asia for its dry seed as a source of protein and carbohydrate as well as for sprouting as vegetable. Besides being one of the shortest duration field crops in the world (can be harvested within two months), soil rhizobium bacteria around the mungbean root zone can symbiotically fix N_2 gas from the air and this makes it among the most popular components in cropping systems. However, seed yield in farmers' fields is still low, varying from 0.3 to 2.1 t/ha with the norm of 0.5-0.7 t/ha (Herridge *et al.*, 2005). Pure line breeding strategies such as pedigree selection, bulk selection, single seed descent and early generation testing have repeatedly showed a limited success in increasing seed yield in legume crops (Saxena *et al.*, 2009). One way to break the yield plateau is to produce hybrid seed to utilize heterosis or hybrid vigor inherently available in living organisms. Heterosis is a well-known phenomenon in which hybrid progeny show superiority compared with their parents (Shull, 1908). There is a long dramatic history of success in hybrid maize, a cross-pollinating crop (Duvick, 1999). In a self-pollinating crop, Yuan (1997) reported a success story in developing hybrid rice, which has recently secured food sustainment in China and India. The success in hybrid rice encouraged mungbean breeders to explore the possibility of developing hybrid cultivars to boost seed yield. The exploitation of heterosis in hybrid cultivars remains the best approach to maximize yield and yield stability. However, a stable cytoplasmic male sterility (CMS) system for hybrid seed production is not yet available in mungbean, although ongoing research is being conducted to develop such a system (Sorajjapinun and Srinives, 2011).

Genetic difference between the inbred or pure line parents presumably contributes to the genetic basis of heterosis. Farooq and Azam (2002) reviewed the uses of molecular markers is measuring genetic distance and heterosis in plant breeding. Schrag *et al.* (2010) applied joint analy-

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ses of hybrids and parental inbred lines for prediction of performance of untested hybrids in maize. In mungbean, Sangiri *et al.* (2007) examined a set of 615 cultivated, wild and weedy mungbeans by using simple sequence repeat (SSR) markers and found that the markers divided the germplasm into distinct sets according to their genetic distances and origins. Their information can be utilized to compare the performance of hybrids derived from the parents with different degrees of genetic distance. The heterotic patterns detected from various parental lines will be useful to the plant breeder to make cross combinations only from promising parents. This approach will help to reduce the number of hybrids entered into various yield trials.

Several methods have been developed to predict hybrid performance in maize using genetic markers (Dudley *et al.*, 1991; Frisch *et al.*, 2010; Maenhout *et al.*, 2010; Schrag *et al.*, 2010; Steinfath *et al.*, 2010). Information from molecular markers was also used to assign new germplasm to heterotic pools in maize (Reif *et al.*, 2003). Xu *et al.* (2004) could not predict yield heterosis in maize, although SSR markers showed high polymorphism among the parental inbreds, whereas Liu and Wu (1998) showed that SSR marker technology could be used to identify heterotic patterns of the parental lines in hybrid rice production. High heterotic effects were obtained from hybrids of genetically diverse parental plants through analysis of RAPD markers (Pirkhezri *et al.*, 2010). However, there has been no report so far in using SSR markers to predict hybrid performance in legumes. Recently, SSR markers have been developed to detect genetic diversity in mungbean (Sangiri *et al.*, 2007; Somta *et al.*, 2009). The variation in SSR markers together with morphology can be used to form hybrid combinations with distinct heterotic patterns in mungbean. It can be a key to the success in breeding for hybrid mungbeans.

The objectives of this study were to assess the heterotic pattern of F_1 hybrids and generate heterotic groups in a set of mungbean based on parental distance in morphology and SSR markers.

2. Materials and Methods

2.1 Plant materials

Five parental mungbean lines were chosen based on genetic distance by SSR markers from the previous study on genetic diversity of mungbean germplasm (Sangiri *et al.*, 2007). The Tthree of them were cultivated mungbean accessions (*V. radiata* var. *radiata*), viz. C357, H192 and Sukhothai (SKT), and while two of them were wild accessions (*V. radiata* var. *sublobata*), viz TC1965 and W166. They were chosen based on their genetic distance as revealed by morphology and SSR markers (Sangiri *et al.*, 2007). All mungbean accessions were obtained from Gene bank, National Institute of Agrobiological Science, Japan. The experiment was conducted from January 2009 to December 2010 at a field of Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand. The field is located at latitude 14° 1' 48.54" N, longitude 99° 57' 51.67" E with an elevation of 10 m above sea level. In the first year, the F_1 hybrid seeds were produced by hand-pollination, using Thai indigenous variety, Sukhothai, as the female parent and crossed with male parents with different degrees of genetic distance, i.e. H192 (close distance), C357 (moderate distance), TC1965 (high distance) and W166 (very high distance). Four F_1 hybrid combinations were produced, viz. SKTxC357, SKTxH192, SKTxTC1965 and SKTxW166. In the second year, all hybrids and their parents were arranged in a randomized complete block (RCB) design with two replications. Each F_1 hybrid and parents were sown in a plot of 5-meter rows, 2 rows per plot, with an equal spacing of 50 cm between plants and between rows. This planting density is lower than that recommended for a production field in order to reduce competition between vigorous F_1 plants in the field. The field management followed normal agricultural practices for mungbean (Park, 1978). Insecticides (cypermethrin, abamectin, chlorfluazuron and omethoate) were alternately sprayed at the manufacturers' recommended rates. Weeds were controlled by plastic mulching and hand-weeding. True hybrid plants were confirmed by comparing morphological characters, days to first flowering and SSR markers with the parents. At maturity, individual plants were hand-harvested to observe the number of branches per plant, clusters per plant, pods per cluster, pods per plant and seeds per pod. Pod length (cm), 100-seed weight (g) and number of seeds per plant were also observed. Data were analyzed based on individual plants as well as plot means.

2.2 DNA extraction and SSR analysis

Total genomic DNA from each of the parental lines and F_1 hybrids were isolated from young leaves using the method modified from Dellaporta *et al.* (1983). Each polymerase chain reaction (PCR) was carried out in a total volume of 10 µl containing 2 ng of DNA, 1×Taq buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 1 unit Taq DNA polymerase (Fermentas) and 5 pmol of each forward and reverse SSR primers. The PCR was performed under the following conditions: 94°C for 2 min, followed by 35 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 10 min in a GeneAmp® PCR System 9700 (Applied Biosystems). Then, the PCR products were run on 4.5% denaturing polyacrylamide gel and visualized by a silver staining solution.

Nineteen SSR primers as reported by Sangiri *et al.* (2007) were used to detect polymorphism between the parental lines and their progenies. They were CEDG013, CEDG015, CEDC050, CEDG056, CEDG075, CEDG087, CEDG 088, CEDG100, CEDG108, CEDG139, CEDG149, CEDG150, CEDG174, CEDG191, CEDG247, CEDG264, CEDG269, CEDG 304 and CEDG305.

2.3 Statistical analysis

The data were analyzed based on their sources of variation (ANOVA) using the R program version 2.10 for Windows (Venables *et al.*, 2009). When an F-test was declared significant, the treatment means were compared by Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

Heterosis and heterobeltiosis of each agronomic trait were calculated in percentage as follows:

$$\% \text{ Heterosis (H)} = [(F_1\text{-mid parent})/\text{mid parent}]x100$$

$$\% \text{ Heterobeltiosis (Hb)} = [(F_1\text{-high parent})/\text{high parent}]x100$$

Significance of H and Hb were tested against their standard errors following the method advocated by Soehendi and Srinives (2005).

Agronomic data were standardized to the mean of zero and variance of one before being used in similarity analyses by Euclidean distance and clustered by an Unweighted Pair Group Method with Arithmatic Mean (UPGMA) (Sneath and Sokal, 1973). A cluster analysis was performed based on similarity between two samples, and distances can be represented by difference between transformed values of the samples.

Molecular data were obtained by scoring amplicons of PCR product as present (1) or absent (0) and entered in the form of a binary data matrix. Then, the Dice coefficient of similarity was calculated and a dendrogram on genetic similarity was constructed. The computer package NTSYS-PC Version 2.2 (Rohlf, 1998) was used for molecular cluster analysis.

Regression analysis was used to explain relationships between genetic distances based on SSR and agronomic traits with the heterosis and heterobeltiosis values. Scatter plots were used to visually identify the relationships between the variables and also estimate regression equations. Coefficient of determination (R^2) was used to test the reliability of the regression models. An R^2 closer to 1.00 revealed more reliability of the regression equation in predicting a dependent variable (i.e. heterosis and heterobeltiosis in this case).

3. Results

3.1 Cluster analysis based on molecular data and agronomic traits

The results showed that SSR markers divided the parental lines into four groups with different degrees of genetic distance from SKT, where H192, C357, TC1965 and W166 showed close, moderate, high and very high distance, respectively. A dendrogram was constructed to show the relationship between the mungbean accessions and their F_1 progenies (Figure 1). In this study, SKT was used as the common female parent and as the reference genotype to measure genetic distance among the parents and their hybrids and then group them. The entries could be divided into three groups. The first group consisted of the cultivated mungbeans with closer genetic distances, viz. SKT, C357 and H192 as well as their F_1s, i.e. SKTxH192 and SKTxC357. The second group included the wild mungbean from Australia, TC1965, with high genetic distance from SKT. Their F_1 hybrid, SKTxTC1965, also fell into this group. The third group, showing the highest genetic distance from SKT, consisted of W166, a wild mungbean from Myanmar and the F_1, SKTx W166.

When agronomic traits were considered, the dendrogram showed three distinct groups. The first group comprised the cultivars SKT, C357 and H192 with their hybrids (Figure 2). The second group consisted of both hybrids between the cultivated and wild accessions, SKTxTC1965 and SKTx W166, which showed blending of characters among their parents. The third group comprised the two wild parents, TC1965 and W166, which showed the highest morphological distance from SKT. A heterotic pattern could be established from the above information consisting of two groups. The first pattern comprised the hybrids between the cultivars, SKTxC357 and SKTxH192 while the second pattern com-

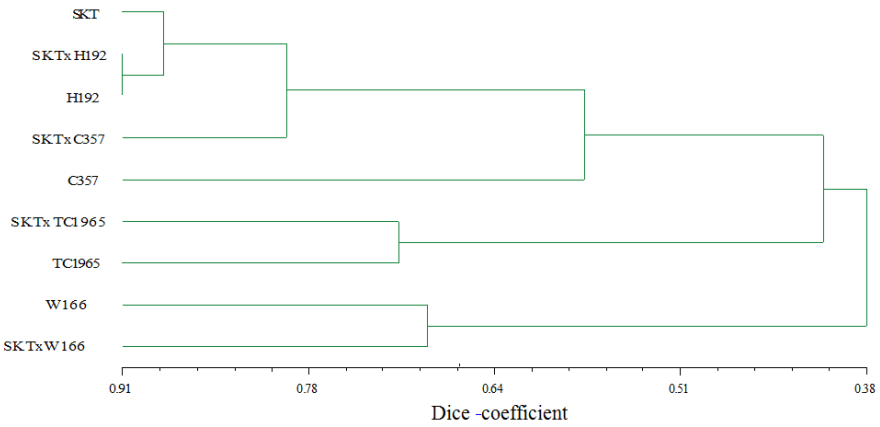

Figure 1. A dendrogram showing relationship between five mungbean accessions and their F_1 progenies based on unweighted pair group method with arithmatic mean (UPGMA) of Dice-similarity coefficients of their SSR markers.

Table 1. Mean comparison and significant test on the percentages of heterosis (H) over mid-parent and heterobeltiosis (Hb) over high-parent in yield, yield components and agronomic traits of four F_1 mungbeans and their parental lines.

Entry	Days to first flowering	No. of branches per plant	No. of clusters per plant	No. of pods per cluster	Pod length (cm)	No. of pods per plant	No. of seeds per pod	100-seed weight (g)	Yield per plant (g)
SKT	38.4	4.7b	26.3	3.78b	14.9a	103.7b	15.3a	8.3a	91.7b
H192	36.2	5.2ab	30.2	9.86a	7.2c	302.4a	11.2c	3.6c	80.7b
SKT x H192 (F_1)	37.0	5.8a	30.6	9.38a	9.5b	283.8a	13.3b	5.1b	130.7a
H	*-0.75ns*	*16.55***	*8.19ns*	*32.54***	*-13.89***	*39.76***	*0.30ns*	*-14.65***	*51.65***
Hb	*-3.54ns*	*11.11ns*	*1.18ns*	*-4.78*	*-36.01***	*-6.16ns*	*-13.28***	*-38.64***	*42.56***
SKT	38.4	4.7a	26.3	3.78b	14.9a	103.7b	15.3a	8.3a	91.7a
C357	33.6	3.0b	27.8	3.80b	6.7c	105.4b	9.8c	4.2c	34.3b
SKT x C357 (F_1)	35.0	4.8a	28.7	7.23a	9.2b	205.1a	12.5b	5.7b	103.3a
H	*-2.72ns*	*23.87***	*6.01ns*	*90.77***	*-14.68***	*96.17***	*-0.92ns*	*-9.36***	*64.01***
Hb	*-8.75**	*1.35ns*	*9.06ns*	*90.26***	*-38.21***	*94.60***	*-18.65***	*-31.6***	*12.69ns*
SKT	38.4b	4.7c	26.3b	3.78a	14.9a	103.7b	15.3a	8.3a	91.7b
TC1965	53.6a	7.4a	129.9a	0.65c	4.4c	81.3b	9.4b	0.9c	5.4c
SKT x TC1965 (F_1)	35.9b	6.1b	136.0a	8.59a	6.0b	1148.2a	7.5c	2.8b	169.2a
H	*-21.9***	*0.47ns*	*74.2***	*30.02***	*-37.50***	*1141.30***	*-39.17***	*-37.45***	*248.69***
Hb	*-32.99***	*-17.88***	*4.73ns*	*-23.81***	*-59.52***	*1007.08***	*-50.95***	*-65.27***	*84.58***
SKT	38.4b	4.7b	26.3b	3.78b	14.9a	103.7b	15.3a	8.3a	91.7b
W166	54.2a	6.2a	117.0a	0.19c	3.7c	22.2c	6.5c	1.5c	1.5c
SKT x W166 (F1)	38.3b	5.8ab	117.7a	5.54a	7.0b	547.2a	10.6b	3.1b	144.9a
H	*-17.21***	*6.41ns*	*64.24***	*179.08***	*-24.16***	*768.80***	*-3.19ns*	*-37.69***	*211.01***
Hb	*-29.34***	*6.67ns*	*0.57ns*	*46.56***	*-52.72***	*427.57***	*-31.04***	*-63.17***	*58.06***

Entry means of each trait in each cross followed by the same letters are not significantly different as determined by DMRT at $P \leq 0.05$. T-tests for heterosis (H) and heterobeltiosis (Hb) were significant at $P \leq 0.05$ (*), significant at $P \leq 0.01$ (**) and ns = non-significant.

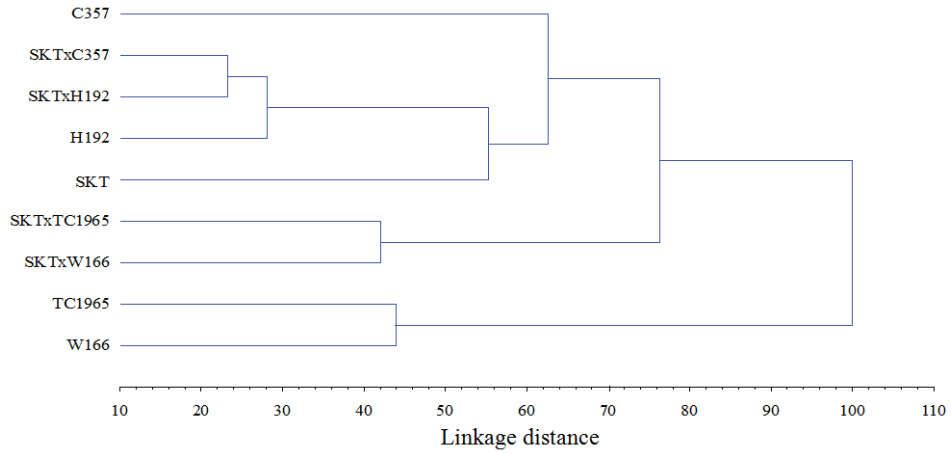

Figure 2. A dendrogram showing relationship between fives mungbean accessions and their F_1 progenies based on unweighted pair group method with arithmatic mean (UPGMA) of Euclidean distances of their agronomic traits.

prised the hybrids of the cultivated and wild mungbeans, SKTxTC1965 and SKTxW166.

3.2 Performance of the hybrids and heterosis:

F_1 hybrids in this experiment showed significant heterosis over mid- and better-parents in almost all characters (Table 1). The cultivated parents and hybrids were earlier in flowering (33-38 days after planting) as compared to wild accessions (53-54 days after planting). Most hybrids had higher yield per plant than their parental lines. The highest seed yield was obtained from the crosses between cultivar and wild accessions SKTxTC1965 and SKTxW166, yielding 169.2 and 144.9 grams per plant, respectively, while the F_1s

from the cultivated accessions, SKTxH192 and SKTxC357 yielded 130.7 and 103.3 grams per plant, respectively. Number of pods per plant showed high heterosis in the same pattern of crosses from which SKTxTC1965 and SKTxW166 gave 1148.2 and 547.2 pods per plant, giving heterobeltiosis at 1007.08% and 427.57%, respectively. The other yield-related traits, viz. 100-seed weight, number of seeds per pod, and pod length tended to express near to either one parent or mid-parent. Thus the useful heterosis in this study came from higher number of pods and seed yield per plant.

Among the cultivars crossed, SKT is more similar to H192 than C357 in agronomic traits, especially days to first flowering, number of branches, number of clusters and seed yield. Their similarity was also confirmed by SSR clustering. The heterosis percentage in seed yield per plant varied from 51.65% and 64.01% in the cultivar crosses SKTxH192 and SKTxC357 to 211.01% and 248.69% for cultivar x wild crosses SKTxW166 and SKTxTC1965, respectively (Table 1). Likewise, the heterobeltiosis in seed yield per plant from the crosses between SKT with the cultivars C357 and H192 gave the yield advantage of only 12.69% to 42.56% over the better parents, while the crosses with wild mungbeans, TC1965 and W166 showed 58.06% to 84.58% over the better parent, respectively. The hybrids showed less number of seeds per pod and 100-seed weight, but more pods per plant. Thus number of pods per plant is the major trait contributing to hybrid vigor in seed yield per plant in this study. Means and heterosis of the other agronomic traits revealed that the hybrids were early in flowering, higher in number of branches and clusters per plant, and shorter in pod length. Although number of clusters per plant was not much different between the parents and the hybrids, number of pods per cluster of the hybrids showed high vigor over their parents.

F_1 crosses obtained from the parents with wider relationship (i.e. between cultivars and wild mungbeans) generally gave higher H and Hb than those obtained from the cultivar crosses. Ranges of hybrid vigor were also higher in the wider crosses as compared to the narrower crosses. H and Hb of the cultivar crosses varied from 96.17% in H of number of pods per plant to -38.64% in Hb of 100 seed weight of SKTxH192. While crosses between the cultivar and wild accessions gave the lowest value of Hb at -65.27% for 100-seed weight and the highest value of H at 1141.30% for number of pods per plant (Table 1). A negative heterosis was obtained for seed size in all crosses when compared to the better parent.

3.3 Relationship between genetic distance and hybrid performance

Relationship between H, Hb and the observed agronomic traits of the four F_1 hybrids are presented in Table 2. H of yield per plant showed positive correlation with number of clusters and number of pods per plant, but showed negative correlation with days to first flowering at the correlation coefficients (r) of 0.996, -0.991 and -0.999 (df = 2), respectively. Thus number of clusters and pods per plant are considered major contributors to heterosis in seed yield of hybrid mungbean in this study. In contrast, H of 100-seed weight, another yield component, is negatively correlated with H of seed yield per plant (r = -0.968) and number of clusters per plant (r = -0.986). Small seed size and high pod number are unique characters of wild mungbean and strongly inherited to their hybrid progenies. For phenotypic correlation, number of pods per plant was negatively correlated with number of seeds per pod (r = -0.974), while pod length was positively correlated with 100 seed weight (r = 0.950).

Heterotic patterns of the four F_1 hybrids are also presented graphically (Figure 3), showing the relationship between genetic distances based on both SSR and phenotypes with the H and Hb values. The genetic distance and hybrid yield performance as measured from H and Hb showed R^2 of 0.78 to 0.99 and could be classified into two groups (Figure 3). The first group comprises SKTxW166 and SKTx TC1965 at the left end of regression line, and the other group comprises SKTxC357 and SKTxH192 at the right end of the regression line. Grouping by SSR markers (Figure 3a and 3c) agreed well with grouping by agronomic traits (Figure 3b and 3d).

4. Discussion

Although hybrid varieties have been developed in maize (Reif *et al.*, 2003), in wheat (Corbellini *et al.*, 2002) and in rice (Riday *et al.*, 2003) based on genetic diversity, heterosis and molecular technique, little research has been conducted in mungbean on heterosis, heterotic grouping and the criteria for selecting parents using molecular markers. Identication of good combiners from diverse germplasm is essential in heterosis breeding. This experiment showed that agronomic characters as well as SSR markers were efficient in classifying heterotic groups, and confirmed the previous study by Chen *et al.* (2003), Sawale *et al.* (2003) and Soehendi and Srinives (2005) that mungbean has a high level of heterosis. In this study, although it would be difficult to choose a high yield hybrid with desirable traits from cultivar x wild crosses, heterosis in number of pods per plant was higher in the hybrids with the wild accessions than with the cultivated ones. In rice, Luo *et al.* (2011) reported that genetic diversity in wild rice showed a great potential for hybrid rice breeding. Thus in mungbean, this relationship gave the breeder a major question on how to compromise between the desirable traits during the formation of hybrids from a given set of parental mungbeans.

In earlier studies, Soehendi and Srinives (2005) reported a mungbean hybrid with the maximum heterosis in seed yield up to 78.5% over the better parent, as compared to hybrid rice that possessed a yield advantage of 10 15 - 20% over the best pure line varieties (Virmani *et al.*, 2003), and its commercial production has recently been successful in China (Yuan, 1997). In mungbean Chen *et al.* (2003) reported several lines that produced F_1 with significant heterosis (H) over

Table 2. Correlation coefficients among heterosis (H), heterobeltiosis (Hb) and phenotypes of yield, yield components and agronomic characters among four F_1 mungbeans.

Characters	Estimators	No. of branches per plant	No. of clusters per plant	No. of pods per cluster	Pod length (cm)	No. of pods per plant	No. of seeds per pod	100-seed weight (g)	Yield per plant (g)
Days to first flowering	H	0.931	-0.992**	-0.893	0.944	-0.994**	0.764	0.958*	-0.999**
	Hb	0.941	-0.301	-0.458	0.987*	-0.903	0.919	0.950*	-0.804
	Phenotypes	0.575	0.361	-0.497	-0.219	0.011	0.023	-0.502	0.358
No. of branches per plant	H		-0.956*	-0.806	0.914	-0.944	0.762	0.959*	-0.938
	Hb		-0.601	-0.730	0.957*	-0.962*	0.975*	0.820	-0.733
	Phenotypes		0.712	0.124	-0.650	0.719	-0.591	-0.802	0.929
No. of clusters per plant	H			0.841	-0.918	0.982*	-0.716	-0.986*	0.996**
	Hb			0.944	-0.399	0.631	-0.622	-0.125	0.281
	Phenotypes			-0.367	-0.989*	0.885	-0.919	-0.985*	0.886
No. of pods per cluster	H				-0.977*	0.927	-0.953*	-0.744	0.875
	Hb				-0.511	0.675	-0.683	-0.220	0.241
	Phenotypes				0.310	0.082	0.098	0.357	0.049
Pod length (cm)	H					-0.974*	0.934	0.852	-0.934
	Hb					-0.956*	0.964*	0.950*	-0.868
	Phenotypes					-0.922	0.964	0.950*	-0.867
No. of pods per plant	H						-0.828	-0.940	0.991**
	Hb						-0.999**	-0.848	0.865
	Phenotypes						-0.974*	-0.845	0.922
No. of seeds per pod	H							0.618	-0.745
	Hb							0.853	-0.848
	Phenotypes							0.853	-0.848
100-seed weight (g)	H								-0.968*
	Hb								-0.913
	Phenotypes								-0.914

*, ** represent a significance at the 0.05 and 0.01 probability levels, respectively (*df* = 2).

mid-parent, and heterobeltiosis (Hb) over high parent in seed yield. Similarly, Sawale *et al.* (2003) reported a significant heterosis over mid- and better-parents in seed yield and yield components. The greatest heterosis in mungbean was obtained from the F_1 whose parental genotypes showing the highest level of divergence in phenotypic characters (Ramanujam *et al.*, 1974).

In this study, clustering based on SSR markers (Figure 1) gave a similar relationship between hybrids and parental lines as clustering by phenotype (Figure 2), although phenotypic clustering showed different relationship between parents and their F_1s. For example, morphological clustering grouped SKTxC357 and SKTxH192 together with H192, but rather far from C357 which was another non-recurrent parent. Similarly, morphological clustering of crosses having wild mungbeans as non-recurrent parents grouped the hybrids SKTxTC1965 and SKTxW166 together but far from their parents (Figure 2), since most traits of the hybrids fell between their parents. Some molecular foundation of phenotypic diversity may reside in the variability of gene and marker expression, which would be related to hybrid vigor. Teklewold and Becker (2006) reported that parents with low molecular distance also had low phenotypic distance, but parents with high molecular distance had either high, intermediate or low phenotypic distance. The SSR clustering revealed a clear genetic relationship between the cultivars and their F_1s by putting them in the same cluster, where SKTxTC1965 was near to TC1965 and SKTxW166 was near to W166 (Figure 1), which is reasonable in terms of SSR grouping. Similarity, Mohammadi *et al.* (2008) revealed that SSR relationship between parental lines could be used to predict performance of maize hybrids. Later, Schrag *et al.* (2010) used AFLP and SSR markers for joint analyses of hybrids and parental inbreds to predict performance of un-

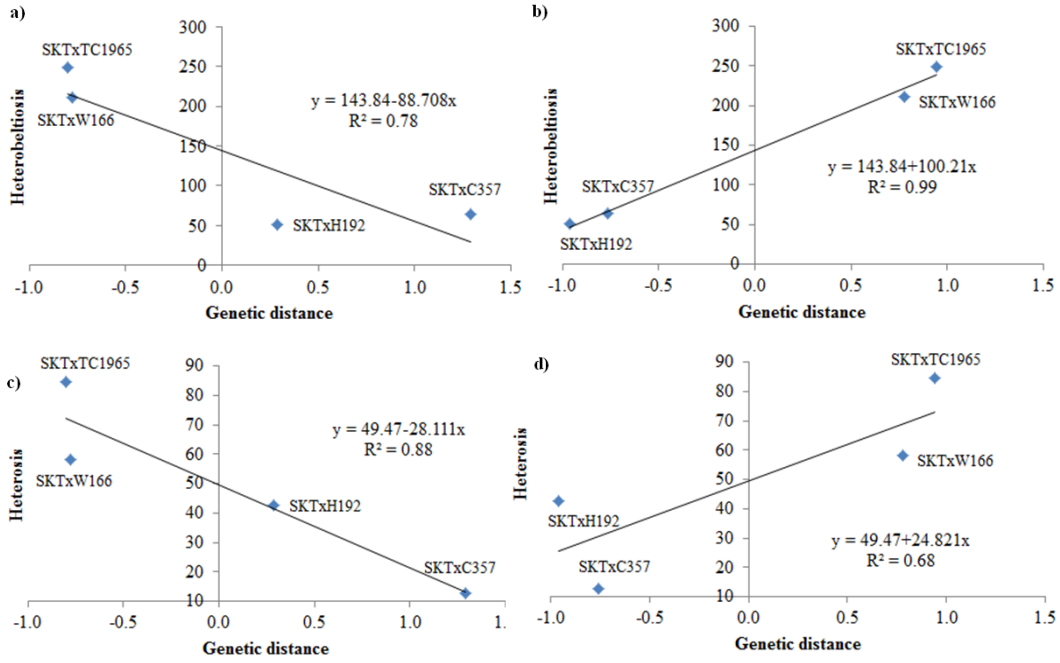

Figure 3. Relationship between genetic distance based on SSR (a, c) and phenotype (b, d) with the heterobeltiosis and heterosis in yield per plant of four F_1 hybrid mungbeans.

tested in maize hybrids. This marker-assisted parental selection consumed less time and money, and was less influenced by environmental factors as compared to conventional identification of combining ability based on morphological characters. However, both methods complemented each other in facilitating mungbean breeders because this crop has rather limited variation in both morphological and molecular markers (Karuppanapandian *et al.*, 2006; Yimram *et al.*, 2009).

Due to a limitation on number of markers and number of hybrid seeds obtained by hand-pollination in mungbean, Kumar *et al.* (2003) classified two major groups of mungbean by RAPD primers and forecasted hybrid vigor by crossing between those with high genetic distance. However, they could not prove this hypothesis. Our research is the first in mungbean to assess parental diversity based on phenotypes and SSR markers and reveal higher vigor from crosses between the parental lines with wider genetic distance. The SSR markers were able to group a large mungbean germplasm and identify the parental lines with promising heterotic patterns rather than evaluating the whole set of germplasm in more expensive field trials.

5. Conclusion

The higher genetic distance between mungbean parents expresses higher degree of heterosis in the F_1 hybrids. Agronomic traits and molecular markers, expressed as phenotypic and genetic distances are useful to classify the parents into heterotic groups and patterns. These can then be used to identify parental lines with high genetic distance and presumably high heterosis for hybrid seed production in mungbean.

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References

Chen, X., Sorajjapinun, W., Reiwthongchum, S. and Srinives, P., 2003. Identification of parental mungbean lines for production of hybrid varieties. Chiang Mai University Journal. 2, 97-105.

Corbellini, M., Perenzin, M., Accerbi, M., Vaccino, P. and Borghi, B., 2002. Genetic diversity in bread wheat, as revealed by coefcient of parentage and molecular markers, and its relationship to hybrid performance. Euphytica. 123, 273-285.

Dellaporta, S.L., Wood, J. and Hicks, J.B., 1983. A plant DNA mini preparation version II. Plant Molecular. Biology. Reporter. 1, 19-21.

Dudley, J.W., Saghai, M.M.A. and Rufener, G.K., 1991. Molecular markers and grouping of parents in maize breeding programs. Crop Science. 31, 718-723.

Duvick, D.N., 1999. Heterosis: feeding people and protecting natural resources. *In* The genetics and exploitation of heterosis in crops. Coors, J.G., Pandey., S. (Eds.). Crop Science Society of America, American Society of Agronomy, Madison, WI, USA. pp: 19-30.

Farooq, S. and Azam, F., 2002. Molecular Markers in Plant Breeding-lll: Practical Applications and Difficulties Encountered. Pakistan Journal of Biological Sciences, 5: 1148-1154.

Frisch, M., Thiemann, A., Fu, J., Schrag, T.A., Scholten, S. and Melchinger, A.E., 2010. Transcriptome-based distance measures for grouping of germplasm and prediction of hybrid performance in maize. Theoretical. and Applied. Genetics. 120, 441-450.

Herridge, D.F., Robertson, M.J., Cocks, B., Peoples, M.B., Holland, J.F. and Heuke, L., 2005. Low nodulation and nitrogen fixation of mungbean reduce biomass and grain yields. Australian. Journal. of Experimental. Agriculture. 45, 269-277.

Karuppanapandian, T., Karuppudurai, T., Sinha, P.B., Kamarul, H.A. and Manoharan, K., 2006. Genetic diversity in green gram (*Vigna radiata* L.) landraces analyzed by using random amplified polymorphic DNA (RAPD). African. Journal. Biotechnology. 5, 1214-1219.

Kumar, S.V., Saw, T.M., Tan, S.G. and Quah, S.C., 2003. Variation of cultivated mungbean and wild *Vigna* as revealed by random amplified polymorphic DNA markers. Pertanika Journal. Tropical. Agricultural. Science. 26, 123-129.

Liu, X.C. and Wu, J.L., 1998. SSR heterogenic patterns of parents for marking and predicting heterosis in rice breeding. Molecular. Breeding. 3, 263-268.

Luo, X., Wu, S., Tian, F., Xin, X., Zha, X., Dong, X., Fu, Y., Wang, X., Yang, J. and Sun, C., 2011. Identication of heterotic loci associated with yield-related traits in Chinese common wild rice (*Oryza rupogon* Griff.). Plant Science. 181, 14-22.

Maenhout, S., Baets, B. D. and Haesaert, G., 2010. Prediction of maize single-cross hybrid performance: support vector machine regression versus best linear prediction. Theoretical. and Applied. Genetics. 120, 415-427.

Mohammadi, S.A., Prasanna, B.M., Sudan, C. and Singh, N.N., 2008. SSR heterogenic patterns of maize parental and prediction of hybrid performance. Biotechnology & Biotechnological Equipment. 22, 541-547.

Park, H.G., 1978. Suggested Cultural Practices for Mungbean. International Cooperator's Guide. Asian Vegetable Research and Development Center. Shanhua, Taiwan. pp 1-4.

Pirkhezri, M., M.E. Hassani, M.E. and Hadian, J., 2010. Genetic diversity in different populations of *Matricaria chamomilla* L. growing in Southwest of Iran, based on morphological and RAPD markers. Research Journal of Medicinal Plant. 4, 1-13.

Ramanujam, S., Tiwari, A.S. and Mehra, R.B., 1974. Genetic divergence and hybrid performance in mungbean. Theoretical. and Applied. Genetics. 45, 211-214.

Reif, J.C., Melchinger, A.E., Xia, X.C., Warburton, M.L., Hoisington, D.A., Vasal, S.K., Beck, D., Bohn, M. and Frisch, M., 2003. Use of SSRs for establishing heterotic groups in subtropical maize. Theoretical. and Applied. Genetics. 107, 947-957.

Riday, H., Charles, B.E., Austin, C.T., Luth, D. and Cazcarro, P.M., 2003. Comparisons of genetic and morphological distance with heterosis between *Medicago sativa* subsp. *Sativa* and subsp. *Falcata*. Euphytica. 131, 37-45.

Rohlf, F.J., 1998. NTSYS-PC Numerical Taxonomy and Multivariate Analysis system. Exeter Software, New York.

Sangiri, C., Kaga, A., Tomooka, N., Vaughan, D.A. and Srinives, P., 2007. Genetic diversity of the mungbean (*Vigna radiata*, Leguminosae) gene pool based on microsatellite analysis. Australian. Journal. of Botany. 55, 837-847.

Sawale, V.S., Patil, J.V., Deshmukh, R.B. and Kute, N.S., 2003. Heterosis and inbreeding depression for yield and its components in mungbean. Legume Research. 26, 134-136.

Saxena, K.B., Kumar, R.V., Chopde, V.K., Srivastava, R.K., Gowda, C.L.L., Rao, K.P.C. and Bantilan, C., 2009. ICPH 2671 the world's first commercial grain legume hybrid reaches farmers. *Proceedings of the* 14th Australian plant breeding and 11th SABRAO conference, Cairns, Australia., August 9-14, 2009.

Schrag, T.A., Möhring, J., Melchinger, A.E., Kusterer, B., Dhillon, B.S., Piepho, H.P. and Frisch, M., 2010. Prediction of hybrid performance in maize using molecular markers and joint analyses of hybrids and parental inbreds. Theoretical. and Applied. Genetics. 120, 451-461.

Shull, G.H., 1908. The composition of a eld of maize. Reports of the Americannn. Breeders. Association. Rep. 4, 296-301.

Sneath, P.H.A. and Sokal, R.R., 1973. Numerical Taxonomy: the principles and practice of numerical classification. San Francisco, Freeman. pp. 188-308.

Soehendi, R. and Srinives, P., 2005. Significance of heterosis and heterobeltiosis in an F_1 hybrid of mungbean [*Vigna radiata* (L.) Wilczek] for hybrid seed production. SABRAO Journal. of Breeding. and Genetics. 37, 97-105.

Somta, P., Sommanas, W. and Srinives, P., 2009. Molecular diversity assessment of AVRDC-The World Vegetable Center elite-parental mungbeans. Breeding. Science. 59, 149-157.

Sorajjapinun, W. and Srinives, P., 2011. Chasmogamous mutant, a novel character enabling commercial hybrid seed production in mungbean. Euphytica. 181, 217-222.

Steinfath, M., Gärtner, T., Lisec, J., Meyer, R., Altmann, T., Willmitzer, L. and Selbig, J., 2010. Prediction of hybrid biomass in *Arabidopsis thaliana* by selected SNP and

metabolic markers. Theoretical. and Applied. Genetics. 120, 239-247.

Teklewold, A. and Becker, H.C., 2006. Comparison of phenotypic and molecular distances to predict heterosis and F_1 performance in Ethiopian mustard (*Brassica carinata* A. Braun). Theoretical. and Applied. Genetics. 112, 752-759.

Venables, W.N., Smith, D.N. and The R Development Core Team., 2009. An Introduction to R: A programming environment for data analysis and graphics version 2.10.1. Bristol Network Theory Ltd.

Virmani, S.S., Sun, Z.X., Mou, T.M., Jauharali, A. and Mao, C.X., 2003. Two-line hybrid rice breeding manual. IRRI, Los Bonos, Philippinines. pp 1-4.

Xu, S.X., Liu, J. and Liu, G.S., 2004. The use of SSRs for predicting the hybrid yield and yield heterosis in 15 key inbred lines of Chinese maize. Hereditas. 141, 207-215.

Yimram, T., Somta, P. and Srinives, P., 2009. Genetic variation in cultivated mungbean germplasm and its implication in breeding for high yield. Field Crops Research. 112, 260-266.

Yuan, L.P., 1997. Hybrid rice breeding for super high yield. Hybrid Rice. 12, 1-6.