Identification of Parental Mungbean Lines for Production of Hybrid Varieties

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ABSTRACT

Five mungbean (Vigna radiata (L.) Wilczeck) varieties from China (CM1 to CM5), two from Thailand (KPS1 and KPS2), and one from Korea (K7) were used as parents to produce 34 F_1s. Hybrids were evaluated along with their parents in the field of the Asian Regional Center of the Asian Vegetable Research and Development Center (ARC-AVRDC), Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand. The hybrid vigor was determined from both heterosis and heterobeltiosis for major agronomic characters, including yield and yield components. Considering overall characters, the superior F_1s were CM5 x K7, CM4 x K7, CM4 x KPS1 and CM3 x K7 while the most promising parental line for future hybrid production was K7. Although K7 per se had low seed yield per plant, its F_1s gave significant heterobeltiosis in most crosses.

Key words: Vigna radiata, Mungbean, Hybrid varieties

INTRODUCTION

The success of hybrid varieties

Whether dominance or overdominance gene action conditions the hybrid vigor, Crow (1952) concluded that there is a decrease of vigor on inbreeding and a gain on outcrossing. Thus, crossing between 2 parental lines carrying diverse genotypes, i.e. each having dominant alleles on different loci should produce a vigorous F_1. With this concept, heterosis has been utilized in commercial hybrid maize production since the 1930s as double cross hybrids (Shull, 1946). He proposed the word heterosis to describe the unusual vigor of the F_1 resulting from hybridization of two inbred lines of maize. Presently, hybrid varieties are produced in economic crops including maize, sunflower, sorghum, cotton, wheat, barley, rice, castorbean, sugar beet, some *Brassica* crops, onion, tomato, etc. Some of these crops are self-pollinating (such as cotton, sorghum, wheat, barley, rice and tomato) but they either have a high rate of random outcrossing or easily cross and produce a fairly large amount of seed that hybrid cultivars can be economically produced (Simmonds and Smart, 1999).

The most recent success story in harnessing hybrid vigor in major crops is hybrid rice in China (Lou and Mao, 1994). The average yield of hybrid rice is more than 2 tons over the conventional pure line varieties. By growing hybrid rice in 70% of the total rice - planting area (~30 Mha), China is able to harvest additional 50 Mt of rice every year. Some paddy field has recently been diverted to grow other crops due to self-sufficiency of this staple cereal in China. While hybrid rice is not so successful in other countries, some breeders in China are exploring to extend the use of hybrid varieties to other crops, including mungbean.

The potential of hybrid mungbean

Mungbean (*Vigna radiata* (L.) Wilczek) is an ancient legume crop with respect to production, trade, and consumption. It is widely grown in countries of South and Southeast Asia, especially China, India, Pakistan, Myanmar, Thailand and Vietnam (Tomooka et al., 2002). In China, it is usually grown for local consumption or exporting in smaller areas as compared to soybean. Cheng et al. (2002) compiled information on potential production of mungbean in China and reported that it had become an economic crop in the provinces of Anhui, Henan, Hebei, Jilin, Jiangsu, Liaoning, Qinghai, Shanxi and Shangdong. The major production areas are in the plain of Northern China and the lower reaches of the Huang and Huai Rivers.

The research work to improve mungbean varieties in China began recently. At present, over 3,000 mungbean accessions have been collected from 20 provinces, preserved and evaluated by the Chinese Academy of Agricultural Sciences, located in Beijing (Cheng et al., 2002). Many varieties are still native lines. They usually give low yield, set pods around the stem with uneven maturity and thus require several harvestings. From 1983, many mungbean varieties were introduced from Asian Regional Center - Asian Vegetable Research and Development Center (ARC-AVRDC) in Thailand to China. These varieties are gradually replacing Chinese native mungbean varieties. Yet, more new cultivars are required to improve average yield of mungbean in China.

With the success in using hybrid rice varieties to boost up the yield in China, a possibility of using hybrid mungbean should be explored. To begin with, combination of crosses should be produced and tested for high heterotic combinations (the crosses with high specific combining ability). If a good cross combination cannot be identified, a parental line that most frequently gives superior F_1s (the line with high general combining ability) should be chosen as a good combiner. Another parents that can specifically combine well with the selected parent can be picked up later.

This study was aimed at identifying parental lines of mungbean for producing superior F_1 hybrids which crossing made from mungbean varieties which are diverse place of origins for possible uses in hybrid mungbean production.

MATERIALS AND METHODS

This study was conducted for 3 successive growing seasons from November, 2002 to July, 2003 in the experimental fields of the ARC-AVRDC, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand. In the first season, five Chinese mungbean varieties (CM1 to CM5) and a Korean variety (K7) were sown in a crossing block to increase seed and observe on their flowering habits under Thailand condition. In the following season, they were planted in the crossing block along with the cultivars KPS1 and KPS2 from Thailand. Altogether 34 F_1 combinations were made as shown in Table 1. At least 10 pods (~80 seeds) were obtained from each cross. In the third season, the F1s were sown in the field along with their parents, each cross was sown in a two-row plot of 4 m long using the spacing of 50 cm between rows and 20 cm between hills with two plants per hill. Insects were controlled by spraying triazophose (Hostathion 40% EC) at the rate of 40 cc per 20 liters of water when the insect population was building up beyond the threshold level. Weeds were controlled by pre-emergence spraying of Imazethapyr at 250 g a.i. per ha. Late weeds were controlled or rogued out by hand weeding.

Table 1. F_1 hybrids obtained from crossing between different mungbean parents (indicated by + sign).

Female \ Male	CM1	CM2	CM3	CM4	CM5	KPS1	KPS2	Korea7
CM1	-	-	-	-	-	+	+	+
CM2	-	-	-	-	-	+	+	+
CM3	-	-	-	-	-	+	+	+
CM4	-	-	-	-	-	+	+	+
CM5	-	-	-	-	-	+	+	+
KPS1	+	+	+	+	+	-	-	+
KPS2	+	+	+	+	+	-	-	+
Korea7	+	+	+	+	+	+	+	-

Data from the following characters were collected:

1. Days to first flowering and harvesting, averaged across each plot
2. Plant height at maturity (cm)
3. Number of pods/plant
4. Number of seeds/pod, averaged from 20 random pods
5. 1,000-seed weight (g), weighed from at least 200 seeds
6. Yield per plant (g)

Characters no. 2, 3 and 6 were taken from 10 random plants from each plot

For each F_1 cross, heterosis (H) and heterobeltiosis (Hb) which are expressed in percents (%) for a particular trait were calculated as follow:

$$H = (\overline{F}_1 - \overline{MP}) \times 100/\overline{MP}$$
$$Hb = (\overline{F}_1 - \overline{Pi}) \times 100/\overline{Pi}$$

Where $\overline{F}_1$ = mean observation of the F_1 progenies taken from the total of n_1 plants

$\overline{MP}$ = mean observation of both parents taken from $n_2 + n_3$ plants

$\overline{Pi}$ = mean observation of one parent of the cross taken from the total of n_2 plants for P_1, or n_3 plants for P_2

Significances of H and Hb were determined by t-tests as follow:

$$H = \frac{\overline{F}_1 - \overline{MP}}{S_H}$$

$$Hb = \frac{\overline{F}_1 - \overline{P}_i}{S_{Hb}}$$

Where S_H and S_{Hb} were the standard errors of the estimates of H and Hb which could be derived as follow:

$$H = \overline{F}_1 - \left(\frac{\overline{P}_1 + \overline{P}_2}{2}\right)$$

$$= \overline{F}_1 - \frac{\overline{P}_1}{2} - \frac{\overline{P}_2}{2}$$

Using the property of expectation, then

$$\text{Variance of H} = \text{Var}\left(\overline{F}_1 - \frac{\overline{P}_1}{2} - \frac{\overline{P}_2}{2}\right)$$

$$= V\overline{F}_1 + \frac{V\overline{P}_1}{4} + \frac{V\overline{P}_2}{4}$$

$$= \frac{VF_1}{n_1} + \frac{VP_1}{4n_2} + \frac{VP_2}{4n_3}$$

$$= \frac{SSF_1}{n_1(n_1-1)} + \frac{SSP_1}{4n_2(n_2-1)} + \frac{SSP_2}{n_3(n_3-1)}$$

Where $V\overline{F}_1$, $V\overline{P}_1$ and $V\overline{P}_2$ were the variances of the mean of each generation; VF_1, VP_1, VP_2, SSF_1, SSP_1 and SSP_2 were the variances and sums of squares of the specified generation, respectively.

$$\text{Then of } S_H = \sqrt{\text{variance H}}$$

In the same manner, variance of Hb could be obtained from

$$\text{Variance of Hb} = \text{Var}\,(\overline{F}_1 - \overline{P}_i)$$

$$= \frac{VF_1}{n_1} + \frac{VP_i}{n_i}$$

$$= \frac{SSF_1}{n_1(n_1-1)} + \frac{SSP_i}{n_i(n_i-1)}$$

$$\text{and } S_{Hb} = \sqrt{\text{variance Hb}}$$

The degree of freedom (*df*) for each test was obtained by summing up the *df* of each generation participating in the estimate. Thus the *df* for testing H was $(n_1\text{-}1)+(n_2\text{-}1)+(n_3\text{-}1)$, and the *df* for testing Hb was $(n_1\text{-}1)+(n_i\text{-}1)$, i = 2 or 3.

RESULTS AND DISCUSSION

When K7 was used as either female or male parent, its F_1s were earlier in both flowering and ripening dates as compared to their parents (Table 2). Days to flowering and days to harvesting of the F1s tended to fall between mid-parent, indicating that additive gene action was conditioning both traits in this experiment. Thus, K7 which is the earliest variety would always give F_1 with shortest flowering and maturity dates. Its progenies flowered between 32-37 days and matured between 60-64 days. However, there was rather big difference between maturity date among the 34 F_1s. The dates ranged from 60-73 days which were considered early in China but normal in Thailand.

Table 2. Agronomic characters, yield and yield components of 8 mungbean cultivars and their 34 F_1s observed under field condition at Kasetsart University, Kamphaeng Saen Campus, Thailand (2002 - 2003).

Mungbean genotypes	Days to flowering	Days to harvesting	Plant height (cm)	Pods/ plant	Seeds/ pod	Seed wt/ plant (g)
KPS1	39	70	79.6	7.6	8.5	27.9
KPS1xK7	36	65	63.3	11.3	10.9	24.5
KPS1xCM1	39	71	63.9	10.0	9.0	32.5
KPS1xCM2	39	71	58.4	10.0	9.0	27.1
KPS1xCM3	41	72	58.9	6.8	8.8	13.7
KPS1xCM4	39	71	61.0	8.8	7.0	22.1
KPS1xCM5	39	72	54.4	9.0	7.3	25.3
KPS2	39	72	67.1	7.1	9.0	23.0
KPS2xK7	33	65	73.8	11.9	10.5	38.8
KPS2xCM1	39	71	74.9	11.1	11.0	39.8
KPS2xCM2	39	73	79.4	9.2	9.8	34.0
KPS2xCM3	39	72	83.9	6.8	10.4	28.1
KPS2xCM4	39	73	80.5	10.7	9.8	37.5
KPS2xCM5	40	71	78.6	10.1	10.4	37.3
K7 32	60	31.6	8.6	9.0	13.6	
K7x KPS1	36	63	62.8	21.6	10.0	58.0
K7x KPS2	37	64	53.2	16.9	8.5	37.0
K7xCM1	33	62	46.7	14.4	9.5	35.7
K7xCM2	35	61	58.4	17.1	9.0	37.0
K7xCM3	33	62	45.6	6.3	10.0	10.0
K7xCM4	37	64	61.4	12.3	10.8	30.2
K7xCM5	33	60	42.1	6.9	10.6	21.9
CM138	68	69.2	13.5	10.0	35.1	
CM1xKPS1	38	72	63.0	11.9	8.3	30.9
CM1xKPS2	39	73	74.5	13.3	9.3	50.8
CM1xK7	34	64	70.5	22.8	11.0	59.7
CM239	69	68.6	11.6	10.2	41.0	
CM2xKPS1	39	71	50.5	18.1	9.8	76.0
CM2xKPS2	38	70	60.2	13.9	9.1	58.3
CM2xK7	34	64	50.0	15.3	10.6	66.9
CM338	70	68.9	12.3	8.8	41.7	
CM3xKPS1	39	71	6.2	11.4	11.0	46.9
CM3xKPS2	39	72	68.4	11.5	9.4	64.0
CM3xK7	33	63	61.8	26.3	11.0	73.9
CM439	69	59.8	9.4	10.2	25.6	
CM4xKPS1	40	68	67.7	25.4	9.9	78.1
CM4xKPS2	38	69	62.8	10.9	8.2	37.1
CM4xK7	32	61	68.2	25.8	11.8	74.8
CM537	68	64.6	12.2	10.6	51.0	
CM5xKPS1	37	67	73.6	20.5	10.2	81.8
CM5xKPS2	37	67	70.2	15.5	10.2	69.6
CM5xK7	34	66	69.7	36.3	11.0	116.1

For plant height, yield, and yield components, the relationship between parents and F_1s were dependent of crosses (Table 2), indicating that non-additive gene action played a major role in controlling these traits. The calculated values of heterobeltiosis (Hb) and heterosis (H) revealed that the hybrid vigors could be of either positive or negative, and might or might not be significantly different from zero (Table 3).

Table 3. Percent heterobeltiosis, %Hb (against male and female parents) and heterosis, %H (against mid-parent) of seeds per pod, pods per plant and seed weight per plant of 34 F_1 mungbeans observed under field condition at Kasetsart University, Kamphaeng Saen Campus, Thailand (2002-2003).

Mungbean genotypes	Seeds per pod			Pods per plant			Seed weight per plant		
	% Hb over		%H	% Hb over		%H	% Hb over		%H
	female parent	male parent	mid-parent	female parent	male parent	mid-parent	female parent	male parent	mid-parent
KPS1xK7	1.7	1.5	1.6	3.7	3.1	3.4	-0.3	1.1	3.7
KPS1xCM1	-0.5	-1.0	-1.0	2.4	-3.5	-3.5	0.4	-0.3	0.1
KPS1xCM2	-0.3	-1.2	-0.8	2.4	-1.6	0.4	-0.1	-1.4*	-0.7
KPS1xCM3	-0.6	-0.7	-0.7	-0.8	-5.5**	-3.2*	-1.4*	-2.8	-2.1**
KPS1xCM4	-3.5**	-4.0**	-3.8**	1.2	-0.6	0.3	-0.6	-0.3	-0.5
KPS1xCM5	-2.0	-3.0*	-2.5*	1.4	-0.4	-3.2*	-0.3	-2.6**	-1.4*
KPS2xK7	-0.3	-0.6	0.3	4.8	3.3	4.0	1.6*	2.5**	2.0**
KPS2xCM1	0.0	1.2	1.0	4.0	-2.4	-8.9**	1.7**	0.5	1.1
KPS2xCM2	-1.9	-0.6	-1.2	2.1	-2.4	-7.0**	1.1*	-0.7	0.2
KPS2xCM3	-0.4	1.7	0.8	-0.3	-5.5	-18.6**	0.5	-1.4	-0.4
KPS2xCM4	-1.0	0.2	-0.3	3.6*	1.3	-1.9	1.4**	1.2**	1.3**
KPS2xCM5	-0.5	0.5	0.3	3.0*	-2.1	2.0	1.4*	-1.4*	0.0
K7xKPS1	2.3	2.3	2.2	12.9**	13.9**	13.4**	4.4**	3.0**	3.7**
K7xKPS2	-2.9*	-2.6*	-2.0	8.3**	9.8**	9.2**	2.3**	1.4**	1.3**
K7xCM1	1.0	1.2	0.8	5.8	0.9	3.4	1.3*	-0.3	0.2
K7xCM2	-0.5	-0.7	-0.7	8.5**	5.5*	47.0**	2.3**	-0.4	1.0
K7xCM3	1.1	1.7	1.3	-2.3	-6.0	-4.2	-0.4	-3.2**	-1.8**
K7xCM4	0.6	0.8	0.6	2.1	1.3	1.7	4.1**	0.5	1.1
K7xCM5	0.2	-0.1	-0.0	-1.7	-5.3	-3.5	0.8	-2.9**	-1.0
CM1xKPS1	-1.4	-0.9	-1.4	-1.6	4.3	-1.6	-0.9	-0.2	-0.5
CM1xKPS2	0.2	-1.0	-0.0	-2.0	6.2**	-6.7**	1.6*	2.8**	2.2**
CM1xK7	1.7	1.5	1.3	9.3*	14.2**	19.3*	2.5**	4.6**	3.5**
CM2xKPS1	-0.2	0.7	0.2	6.5**	10.5**	8.5**	3.5**	4.8**	4.2**
CM2xKPS2	-1.1	-2.4	-1.8	2.3	6.8**	-2.3	1.7**	3.5**	2.6**
CM2xK7	-0.4	-0.2	-0.4	3.7*	6.7**	7.2**	2.6**	5.3**	4.0**
CM3xKPS1	2.6*	2.7*	2.6*	0.9	3.8	1.4	0.5	1.9**	1.2
CM3xKPS2	-0.4	-2.5*	-1.3	-0.8	4.4	-13.8**	2.2**	4.1**	3.2**
CM3xK7	2.1	1.5	1.7	14.0**	17.7**	16.4**	3.2**	6.0**	4.6**
CM4xKPS1	0.9	1.4	1.2	16.0**	17.8**	16.9**	5.2**	5.0**	5.1**
CM4xKPS2	-0.8	-2.0*	-1.3	1.5	3.8*	-1.7	1.2*	1.4**	1.3*
CM4xK7	2.4*	2.2*	2.2*	16.4**	17.2**	16.8**	4.9**	6.1**	5.5**
CM5xKPS1	0.2	1.2	0.7	8.3**	12.9**	10.6**	3.1**	5.4**	4.2**
CM5xPKS2	0.0	-1.0	-0.2	3.3	8.4**	7.4**	1.8*	4.6**	3.3**
CM5xK7	0.1	0.4	0.2	24.1**	27.7**	25.9	6.5**	10.2**	8.4**

Among the yield components, 1,000-seed weight showed least number of crosses with heterosis. This is because the parental lines used in this study are less diverse in this trait, with the 1,000-seed weight varying between 60-70 gm. Most F1s gave 1,000-seed weight of similar range. When Chinese mungbeans were used as a parent, they gave more hybrid vigor than when KPS2 was used. K7 gave the F1s with moderate vigor between the two afore-mentioned groups. The heterosis of 1,000-seed weight in this experiment was found to be between -40.6% in K7 x CM3 to 19.5% in CM5 x KPS2 while heterobeltiosis ranged from -27.8% in K7 x CM3 to 24% in KPS2 x CM2 (data not shown). More crosses expressed negative hybrid vigor in number of seeds per pod such as KPS1 x CM4, KPS1 x CM5 and K7 x KPS2. Positive vigor in which the F1s showed more seeds per pod than their parents was found in the crosses CM3 x KPS1 and CM4 x K7. Stronger and more positive hybrid vigor was identified in number of pods per plant, especially in the crosses K7 x KPS1, CM1 x K7, CM3 x K7, CM4 x KPS1, CM 4 x K7, CM5 x KPS1 and CM5 x K7. The heterosis and heterobeltiosis found in these crosses were well over 10%, and even over 25% in the last cross. K7 seemed to carry good genes for this trait and thus gave more F1s with significant heterosis than the other parents. A few crosses gave negative heterosis in number of pods per plant such as KPS2 x CM3, CM3 x KPS2, KPS2 x CM1 and CM1 x KPS2. It revealed that KPS2 had contributed negatively to its hybrids in the expression of this trait. Hamid et al. (1996) reported that number of pods per plant was relatively high heritable (heritability = 41.0%) as compared to 31.9% and 21.2% in seeds per pod and seed size respectively.

For seed weight (yield) per plant, high positive heterosis was identified in K7 x KPS1, CM1 x K7, CM1 x KPS2, CM2 x KPS1, CM2 x K7, CM3 x KPS2, CM3 x K7, CM4 x KPS1, CM4 x K7, CM5 x KPS1, CM5 x KPS2 and CM5 x K7. Although the Korean K7 was a major contributor for hybrid vigor, the counterpart parent was equally important as a yield contributor. Hamid et al. (1996) reported higher heritability of yield per plant as compared to the yield components while days to maturity expressed highest heritability (82.1%) among all the traits they studied.

CONCLUSIONS

It can be concluded from this study that K7 should be chosen as a parent to cross with either Thai or Chinese mungbeans in order to produce superior F_1s. The crosses between Chinese and Thai mungbeans did not give high vigorous yield in F_1s cross. Since over 50% of the total mungbean acreage in China are AVRDC-improved varieties/lines, it is likely that the Chinese mungbeans are related to KPS1 and KPS2 which also originated from AVRDC (Cheng et al., 1993; Cheng and Wang, 1998). Thus they did not give F_1 progenies with high heterosis as compared to using K7 as a parent. The hybrid yield with maximum heterosis of close to 10% in the cross CM5 x K7 is not economically feasible to produce commercial hybrid seed of mungbean. More cross combinations should be evaluated for hybrid vigor, coupled with possible means to produce hybrid seed (i.e. male sterile system, hand-pollination, etc.). At the moment, it is still far from reality that hybrid mungbean will be commercialized in a popular manner as in hybrid rice.

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REFERENCES

Cheng X., W. Youtian, and C. Y. Yang. 1993. Compilation of papers on technology and utilization of AVRDC - Improved mungbeans in China. Institute of Crop Germplasm Resources, Beijing, China.

Crow, J.F. 1952. Dominance and overdominance. p. 282-297. In Heterosis. Iowa State College Press, Ames, Iowa, USA.

Hamid, A., A. Hashem, A. A. Miah and M.L. Chadha. 1996. Genotypic variation in morphological and physiological characters and yield attributes of mungbean. p.71-78. In A.N. Asthana and D.H. Kim (eds) Recent advances in mungbean research. Indian Society of Pulses Research and Development, Kanpur, India.

Lou X., and C.X. Mao. 1994. Hybrid rice in China – A Success story. FAO Regional Office, Bangkok, Thailand. 26 pp.

Shull, G.H. 1946. Hybrid seed corn. Science 103 : 547-550.

Simmonds, N.W., and J. Smartt. 1999. Principles of crop improvement. Blackwell Science Ltd., London. 412 pp.

Tomooka, N., D.A. Vaughan, H. Moss, and N. Maxted. 2002. The Asian *Vigna* : Genus *Vigna* subgenus *Ceratotropis* genetic resources. Kluwer Academic Publishers, Dordrecht, The Netherlands. 270 pp.

Cheng X., and W. Suhua. 1998. Evaluation of mungbean germploom resources in China. p 7-10. In X.Cheng, Y. Wang and C. Y. Yang (eds) Proceedings on technology and utilization of mungbean in China. China Agriculture Press, Beijing, China.

Cheng X., T. Yue, and M. Koizumi. 2002. Industrial development and technology utilization of mungbean in China. China Agricultural Science and Technology Press, Beijing, China. 191 pp.