Indian Journal of Agricultural Sciences **83** (9): 907–11, September 2013/Article

Crossability relationship between blackgram (*Vigna mungo*) and ricebean (*V. umbellata*) for successful blackgram × ricebean hybridization programme

R DHIMAN[1], R K MITTAL[2], H K CHAUDHARY[3] and ASHOK KUMAR YADAV[4]

CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh 176 062

Received: 28 January 2013; Revised accepted: 22 March 2013

ABSTRACT

To understand genotypic effects of blackgram [*Vigna mungo* (L.) Hepper], ricebean [*V. umbellata* (Thunb.) Ohwi & Ohasi] genotypes and their interaction in blackgram × ricebean hybridization programme, three diverse genotypes of blackgram were pollinated with six ricebean genotypes in line × tester fashion under glasshouse conditions. The analysis of variance showed significant influence of different crosses on pod formation, healthy hybrid seed formation, seed germination and regeneration. Blackgram and ricebean genotypes indicated their significant influence on almost all the hybridization parameters. The results showed association of both additive and dominance gene action with the preponderance of dominance gene action for all the four parameters. Overall proportional contribution of blackgram × ricebean interaction for all the four parameters was the highest, followed by ricebean genotype in case of pod formation and regeneration, whereas blackgram genotypes were the second major contributor in case of healthy hybrid seed formation and seed germination. On the basis of hybrid formation efficiency (HFE) as well as GCA, PDU 1 among blackgram genotypes and Local and PRR 1 among ricebean genotypes have emerged to be the best general combiners and could be further utilized to accelerate the hybridization programme. Based on significant estimates of positive SCA effects, the cross combination PDU 1 × BRS 2 for pod formation, Palampur 93 × PRR 9301 for healthy hybrid seed formation and germination and the combinations UG 218 × BRS 1 and UG-218 × PRR 1 for regeneration were identified to be the best specific combiners.Results suggest presence of reproductive barriers that leads to introgression difficult and also that the success of the *V. mungo* × *V.umbellata* hybridization programme depends on the genetic interaction amongst different genotypes of both the species.

Key words: Blackgram, Gene action, Genotypic effects, Hybrid frequency, Hybridization, Ricebean

Blackgram [*Vigna mungo* (L.) Hepper] is a tropical leguminous plant, which belongs to the Asiatic *Vigna* species along with *V. radiata*, *V. trilobata*, *V. aconitifolia* and *V. glaberecence* (Sivaprakash 2004). It is extensively cultivated in the Indian subcontinent and to a lesser extent in Thailand, Australia and other Asian and south Pacific countries (Poehlman 1991). Low productivity in this crop is attributable to its narrow genetic base due to common ancestry of various superior genotypes, poor plant type, vulnerability to abiotic and biotic stresses and their cultivation in marginal and harsh environment (Ali *et al.* 2006). Blackgram is susceptible to different leaf spotting pathogens such as *Cercospora canescens*, *Cercospora cruenta* and *Colletotrichum truncatum* in high rainfall areas of the mid-hills in Northwestern Himalayas (Singh *et al*. 1978, Kaushal and Singh 1988). There had been always possibility of improving the crop by incorporating wild genes to the cultivated species. Stepwise utilization of primary, secondary and tertiary gene pools of crop can result in tremendous improvement in yield (Pandiyan *et al.* 2010). Underutilized related species of blackgram, i e ricebean [*V. umbellata* (Thunb.) Ohwi & Ohasi] has been found to be nutritive and resistant to the most of these pathogens and provide a worthwhile opportunity to introgress genes for disease resistance and other desirable traits.Also it is reported to be immune from *Cercospora* leaf spot (Marappa 2008).

For the first time, interspecific hybrids of *Vigna mungo* × *V. umbellata* were classified as a partially compatible cross by Al-Yasiri and Coyne (1966), in which pods collapse in the early stages of development and the reciprocal cross as incompatible in which no pods set. But first successful interspecific hybrids were obtained by Biswas and Dana (1975) by culturing the poorly developed embryonic axes dissected out of the water-soaked seeds. Ahn and Hartmann

[1] Senior Research Fellow (e mail: dhiman.22@gmail.com), [2] Professor (e mail: mittalrk@hillagric.ac.in), [3] Professor (e mail: ctihkc@ rediffmail.com), Department of Crop Improvement; [4] Scientist (e mail: ashok@ihbt.res.in), CSIR-IHBT, Palampur, Himachal Pradesh 176 061

(1978) also successfully obtained wide hybrids of blackgram and ricebean but found it a very difficult combination to produce. Some seedlings obtained by embryo culture from crosses using blackgram as the pistillate parent reached adult stage, but died before flowering. Similarly, varying degrees of success in interspecific hybridization of *Vigna mungo* × *V. umbellata* have been reported by few workers, viz. Rashid (1988), Karmaker *et al.* (1989), Dar *et al.* (1991), Mittal *et al.* (2005, 2008, 2010) and Pal *et al.* (2005). But main causes of failures of interspecific crosses in food legumes are still not fully understood (Bharathi *et al.* 2006)

Hence the present investigation was taken up with the objective of understanding crossability relationship including genotypic influence of blackgram, ricebean and their interaction on blackgram × ricebean hybridization programme.

MATERIALS AND METHODS

Three diverse genotypes of blackgram and six genotypes of ricebean were selected from the Indian gene pool for the present investigation (Table 1). Both blackgram and ricebean genotypes were raised in pots under glasshouse conditions. Staggered sowing was done at an interval of about 12-15 days, so as to get sufficient number of buds for emasculation and flowers for pollination in both the crops simultaneously. This resolved the problem related to variation in flowering time between both the crops, irrespective of number of genotypes involved. Pollination was done using whole anther of the male parent and since there was large population of ricebean genotypes and large number of flowers were available, thus providing equal chance to every emasculated bud of blackgram. Fifty buds of each blackgram genotype were hybridized with each of the six ricebean genotypes in line (blackgram genotype) × tester (ricebean genotype) fashion under glasshouse conditions. No growth hormones or immunosuppressant were used, so as to get unbiased genotypic effects. The crossed pods were harvested after full maturity.

F_1 seeds developed were of two types, viz. highly shriveled, minute, brown coloured and the second was bold and comparatively brown coloured but very weak as compared to the selfed ones. Both type of hybrid seeds were grown under *in vitro* condition on sterilized salt solution as per the composition given by Sangers *et al.* (1959). These F_1 seeds were surface sterilized with 0.02% mercuric chloride for two minutes, washed 3-4 times with sterilized water and then put in sterilized salt solution under laminar airflow. Seeds were kept in an incubator at 25±1°C for 4-5 days. The sterilized salt solution was changed every day under aseptic conditions. Four to five days old imbibed seedlings were transferred to the pots in glasshouse. Since, the first kind of minute hybrid seeds could not germinate in any case, they were not taken into account for working out hybrid seed development frequency.

Observations were recorded with respect to pod formation, total seed formation, healthy hybrid seed formation (HHS), germination and regeneration in each cross. The information was used to generate data with respect to pod formation frequency (Number of pods obtained/100 blackgram flower pollinated with ricebean), total seeds formed/cross, healthy hybrid seed formation frequency (HHS/100 seeds developed), seed germination frequency (Number of seed germination/100 HHS), regeneration frequency (Number of plants developed/100 seeds germinated) and

Table 1 Mean performance of various blackgram and ricebean genotypes for various hybrid induction parameters pooled over all ricebean and blackgram genotypes respectively

Genotypes	Buds pollinated	Pods developed (%)	Total seeds	Healthy hybrid seed (%)	Seed germination (%)	Regeneration (%)	Hybrid formation efficiency (%)
Lines (Blackgram)							
Palampur 93	300	16.67 (50)	110	20.91 (23)	26.09 (6)	50 (3)	1.00
PDU 1	300	19 (57)	165	39.39 (65)	23.08 (15)	46.67 (7)	2.33
UG 218	300	16 (48)	118	40.68 (48)	8.33 (4)	100 (4)	1.33
Mean	300.00	51.67	131.00	45.33	8.33	4.67	1.56
Tester (Ricebean)							
Naini	150	20 (30)	56	46.43 (26)	19.23 (5)	60 (3)	2.00
BRS 1	150	15.33 (23)	61	31.15 (19)	21.05 (4)	75 (3)	2.00
BRS 2	150	12 (18)	46	43.48 (20)	25 (5)	20 (1)	0.67
PRR 1	150	26.67 (40)	137	21.90 (30)	20 (6)	66.67 (4)	2.67
PRR 9301	150	6 (9)	11	54.54 (6)	16.67 (1)	0 (0)	0.00
Local	150	23.33 (35)	82	42.68 (35)	11.43 (4)	75 (3)	2.00
Mean	150.00	25.83	65.50	22.67	4.17	2.33	1.56

Parenthesis value represents original number obtained

Table 2 Line × tester analysis for hybrid induction parameters in blackgram × ricebean crosses

Source of variation	df	Mean sum of squares (MS)			
		Pods developed	Healthy hybrid seed	Seed germination	Regeneration
Crosses	17	1 408.08**	3 476.91**	1 771.73**	599.49**
Blackgram genotypes	2	26.13*	396.57**	373.24**	899.61**
Ricebean genotypes	5	139.10**	142.01**	62	1 148.52**
Blackgram × ricebean interaction	10	110.30**	535.16**	269.70**	1 199.31**
Error	162	7.61	35.86	68.63	54.01

*P=0.05, **P=0.01

hybrid formation efficiency (HFE) (Number of plants developed/ 100 blackgram flower pollinated with ricebean). The mean data of all the crosses with respect to the frequency of pod formation, healthy hybrid seed formation frequency, seed germination frequency and regeneration frequency were subjected to line × tester analysis as per Kempthorne (1957).

RESULTS AND DISCUSSION

The mean frequency of pod formation ranged from 0 to 40% in different crosses, while it was 0 to 66.66 %, 0 to 40% and 0 to 100% for healthy hybrid seed formation, germination and regeneration, respectively.Low percentage of pod set (5.56%) was also observed by Thiyagu *et al.* (2008) and concluded the presence of reproductive barriers that render introgression difficult. Highest pod formation frequency was observed in case of PDU 1 (19%), healthy hybrid seed formation and regeneration frequency in UG-218 (40.68% and 100%), while highest germination frequency was exhibited by Palampur 93 (26.09%). In case of ricebean genotypes, PRR 1 (26.67%) produced highest pod formation frequency, while PRR 9301 (54.54%) and BRS 2 (25%) exhibited highest healthy hybrid seed formation and germination frequency, respectively. Similarly, highest regeneration frequency was observed in BRS 1 (75%) and Local (75%) (Table 1).Pandiyan *et al.* (2012) working on a cross between *Vigna radiata* and *V. trilobata* found crossability percentage to be 10.25 %, while hybrid germination of 34.21% was observed.

Blackgram genotype PDU 1 was found to have highest pod formation frequency (19%) but lowest regeneration frequency (46.67%). Whereas, UG 218 exhibited highest healthy hybrid formation (40.68%) and regeneration frequency (100%) but lowest pod formation (16%) and seed germination (8.33%) frequencies. In case of ricebean, genotype PRR 1 exhibited highest pod formation frequency (26.67%) but lowest healthy hybrid formation (21.90%), while highest healthy hybrid formation (54.54%) was observed in PRR 9301 with no seed germination (Table 1). Frequency of all the four hybrid induction parameters, viz. pod formation, healthy hybrid seed formation, seed germination and regeneration in different crosses of *Vigna mungo* × *V.umbellata* clearly indicated that both blackgram and ricebean genotypes were behaving differently for different crosses.

Analysis of variance indicated that MS due to crosses was statistically significant for all the four parameters (Table 2). The analysis of variance showed significant influence of crosses on all the four parameters. Amongst the blackgram genotypes, significant differences were emanated for all the four parameters, whereas, the ricebean genotypes exhibited differences for pod formation, healthy hybrid seed formation and regeneration. Blackgram × ricebean interaction was also found significant for all the four parameters, indicating the presence of genetic interactions between the lines and the testers for these traits and also involvement of non-additive gene action.

Highest positive general combining ability (GCA) effects were observed in PDU 1 for all the parameters, viz. pod formation (2.36), healthy hybrid seed formation (9.21), seed germination (7.95) and regeneration (12.25) amongst blackgram genotypes (Table 3). Amongst ricebean genotypes, highest positive GCA effects were observed in PRR 1 (7.98) for pod formation, whereas it was highest for healthy hybrid seed formation (11.98), seed germination (6.40) and

Table 3 GCA effects of frequencies of pods developed, healthy hybrid seeds, seed germination and regeneration in blackgram and ricebean genotypes

Genotypes	GCA			
	Pods developed	Healthy hybrid seed	Seed germination	Regeneration
Palampur 93	–1.59*	–6.20*	–0.13	–12.24*
PDU 1	2.36*	9.21*	7.95*	12.25*
UG 218	–0.77*	–3.01*	–7.82*	–0.01
CD (P=0.05)	0.70	1.52	2.10	1.86
Naini	3.02*	–0.03	0.96	3.91*
BRS 1	–0.13	–7.03*	1.97	14.99*
BRS 2	–6.53*	–2.60*	–4.98*	–20.48*
PRR 1	7.98*	–5.30*	1.24	14.99*
PRR 9301	–9.48*	2.97*	–5.59*	–28.38*
Local	5.15*	11.98*	6.40*	14.99*
CD (P=0.05)	0.99	2.14	2.97	2.63

Table 4 SCA effects of frequencies of pods developed, healthy hybrid seeds, seed germination and regeneration in blackgram and ricebean genotypes

Genotypes		SCA			
Female	Male	Pods developed	Healthy hybrid seed	Seed germi- nation	Re- generation
Palampur 93	Naini	10.23*	16.40*	10.48*	33.73*
	BRS 1	–4.64*	–16.57*	–18.10*	–32.09*
	BRS 2	–14.27*	–22.43*	–11.15*	3.38
	PRR 1	10.05*	–19.36*	–17.38*	–32.09*
	PRR 9301	8.55*	26.73*	19.45*	14.15*
	Local	–9.92*	15.23*	16.70*	12.91*
PDU 1	Naini	–1.61	10.36*	–1.08	-0.50
	BRS 1	–1.45	4.36*	1.95	–11.58*
	BRS 2	13.32*	3.06	12.58*	5.46*
	PRR 1	–5.16*	2.08	5.64*	–11.58*
	PRR 9301	–4.14*	1.60	–16.60*	–13.21*
	Local	–0.97	–21.45*	–2.49	31.40*
UG 218	Naini	–8.61*	–26.76*	–9.40*	–33.23*
	BRS 1	6.08*	12.21*	16.16*	43.66*
	BRS 2	0.94	19.38*	–1.43	–8.84*
	PRR 1	–4.89*	17.28*	11.74*	43.66*
	PRR 9301	–4.41*	–28.33*	–2.85	–0.94
	Local	10.89*	6.23*	–14.21*	–44.31*

regeneration (14.99) in the Local genotype. The genotype BRS 1 and PRR 1 also exhibited the same highest positive GCA level (14.99) for regeneration. Highest positive specific combining ability (SCA) effects were observed in the cross PDU 1 × BRS 2 (13.32) for pod formation, Palampur 93 × PRR 9301 for healthy hybrid seed formation (26.73) and germination (19.45), whereas UG 218 × BRS 1 and UG 218 × PRR 1 (43.66) for regeneration (Table 4).

Estimates of variance due to general combining ability (σ^2_{gca}), specific combining ability (σ^2_{sca}), additive genetic variance (σ^2_A) and dominance variance (σ^2_D),exhibited negative estimates of variances for σ^2_{gca} and σ^2_A but positive estimates for $\sigma^2_D$for all the four parameters, viz. pod formation (10.27), healthy hybrid seed formation (49.93), seed germination (20.11) and regeneration (114.53). Negative estimates of variance for σ^2_{gca} and σ^2_A but positive estimates for σ^2_D for all the parameters indicated the preponderance of non-additive genetic control of these parameters. Significant values of GCA for all the parameters in case of blackgram and ricebean, indicated presence of additive genetic control of these parameters. This clearly indicated presence of both additive and non-additive gene action with the preponderance of non-additive genetic control of these parameters.

The degree of dominance $(\sigma^2_D/\sigma^2_A)^{1/2}$ could not be estimated in all the four parameters due to negative values of σ^2_A, that is, –12.31, –118.16, –23.15 and –77.89 for pod formation, healthy hybrid seed formation, seed germination and regeneration, respectively. Overall proportional contribution of blackgram × ricebean interaction for pod formation (59.60), healthy hybrid seed (78.07), seed germination (71.85) and regeneration (61.39) was observed highest, followed by ricebean genotype in case of pod formation (37.58) and regeneration (29.40), while blackgram genotypes were the second major contributor in case of healthy hybrid seed formation (11.57) and seed germination (19.89). This clearly indicates the importance of genetic interactions between the lines and the testers for the related traits.

Based on significant estimates of positive GCA effects, PDU 1 among the blackgram genotypes was identified as outstanding genotype influencing frequency of all the four parameters. In case of ricebean, PRR 1 for pod formation, Local for healthy hybrid seed formation, seed germination and regeneration and the genotypes, BRS 1 and PRR 1 for regeneration were identified to be outstanding genotypes influencing the frequency of respective parameter.

Based on significant estimates of positive SCA effects, the combination, PDU 1 × BRS 2 for pod formation, Palampur 93 × PRR 9301 for healthy hybrid seed formation and germination and UG 218 × BRS 1 and UG 218 × PRR 1 for regeneration were identified to be best the specific combiners.

Conclusively, on the basis of hybrid formation efficiency (HFE) as well as general combining ability (GCA), PDU 1 among blackgram genotypes and Local and PRR 1 among ricebean genotypes have emerged to be the best general combiners. Thus these two ricebean genotypes can be further utilized to accelerate *Vigna mungo* × *V.umbellata* hybridization programme.Results clearly suggests presence of reproductive barriers that leads to introgression difficult and also that the success of the *V. mungo* × *V.umbellata* hybridization programme depends on the genetic interaction amongst different genotypes of both the species.

REFERENCES

Ahn C S and Hartmann R W. 1978. Interspecific hybridization among four species of the genus, *Vigna* Savi. *Proceedings of 1st International Mungbean Symposium*, pp 240–6. Asian Vegetable Research and Development Center, Shanhua, Taiwan, ROC.

Al-Yasiri S A and Coyne D P. 1966. Interspecific hybridization in the genus *Phaseolus*. *Crop Science* **6**: 59–60.

Ali M, Gupta S, Singh B B and Kumar S. 2006. Role of plant introductions in varietal development of pulses in India. *Indian Journal of Plant Genetic Resources* **19**: 346–52.

Bharathi A, Selvaraj K S V, Veerabadhiran P and Laksh B S. 2006. Crossability barriers in mungbean (*Vigna radiata* L. Wilczek): with its wild relatives. *Indian Journal of Crop Science* **1**(1-2): 120–4.

Biswas M R and Dana S. 1975. Blackgram × ricebean cross. *Cytologia* **40**: 787–95.

Dar G M, Verma M M, Ghosal S S and Brar J S. 1991. Characterization of some interspecific hybrids and amphid ploids in *Vigna*. *Proceedings of golden jubilee celebrations symposium* *on legumes*, Indian Society of Genetics and Plant Breeding, pp 73–8.

Kempthorne O. 1957. *An introduction to Genetic Statistics*. Iowa State University Press. Ames.

Karmaker P G, Biswas M R and Dana S. 1989. Meiosis in allotetraploids of *Vigna mungo* × *V. umbellata* cross. *Cytologia* **54**: 79–82.

Kaushal R P and Singh B M. 1988. Interspecific hybridization between urdbean (*Vigna mungo* (L.) Hepper) and adzukibean (*Vigna angularis* (Wild.) Ohwi and Ohashi). *Euphytica* **39**(1): 53–7.

Marappa N. 2008. Screening of mungbean genotypes and its wild relatives for resistant sources to *Cercospora leaf* spot disease. *Asian Journal of Bio Science* **3**(2): 324–6.

Mittal R K, Katna G and Sood B C. 2005. Interspecific hybridization in the genus *Vigna. IFLRV-IV,* October 2005, New Delhi.

Mittal R K, Sood B C, Sharma R and Katna G. 2008. Interspecific hybridization and DNA based polymorphism in urdbean (*Vigna mungo*), ricebean (*V. umbellata*) and adzukibean (*V. angularis*). *Chromosome Science* **11**: 48.

Mittal R K, Sharma R, Gupta D, Dhiman R and Parmar Anu. 2010. Identification of interspecific hybrids of Urd bean x Ricebean and their further evaluation. *The 4th Asian Chromosome Colloquium*, 11-14 October 2010. Beijing, China, pp 135.

Pal S S, Sandhu J S and Inderjit S. 2005. Exploitation of genetic variability in interspecific cross between *Vigna mungo* × *V. umbellata*. *Indian Journal of Pulses Research* **18**: 9–11.

Pandiyan M, Senthil N, Ramamoorthi N, Muthiah A R, Tomooka N, Duncan V and Jayaraj T. 2010. Interspecific hybridization of *Vigna radiata* x 13 wild *Vigna* species for developing MYMV donar. *Electronic Journal of Plant Breeding* **1**: 600–10.

Pandiyan M, Senthil N, Suersh R, Chakravarthy N, Packiaraj D and Jagadeesh S. 2012. Interspecific hybridization of *Vigna radiata* × *Vigna trilobata. Wudpecker Journal of Agricultural Research* **1**(6): 233–4.

Poehlman J M. 1991. History, description, classification and origin. (*In*) *The Mungbean*, pp 6–21.Poehlman J M (Eds.).West View, Boulder.

Rashid K A, Smartt J and Haq N. 1988. Hybridization in genus *Vigna*. *Mungbean: Procedings of second International Symposium*, pp 205–14. Shanmugasundram S (Eds.). Asian vegetable Research and Development Center, Shanhua, Tainan, Taiwan.

Sangers M E, Clifford J F and James G R. 1959. Influence of environmental factors on origin of colchicin-induced true-breeding diploid mutants in sorghum. *American Journal of Botany* **46**: 119–25.

Singh B M, Sood A K and Saharan G S. 1978. Occurrence of leaf spot and blight of blackgram caused by *Colletotrichum dematium*. *Indian Phytopathology* **31**: 100–1.

Sivaprakash K R, Prashanth S R, Mohanty B P and Parida A. 2004. Genetic diversity of blackgram (*Vigna mungo*) landraces as evaluated by amplified fragment length polymorphism markers. *Current Science* **86**: 1 114–6.

Thiyagu K, Jayarnani P, Nadarajan N. 2008. Pollen pistil interaction in inter-specific crosses of *Vigna* sp. *Cytologia* **73**(3): 251–7.