Theor. Appl. Genet. 58, 5-10 (1980)

Are 'Multiple Cross-multiple Pollen Hybrids' an Answer for Productive Populations in *Brassica campestris* (var.) Brown Sarson?

Part 2: Evaluation of 'Mucromphs'

A. Bandyopadhyay and V. Arunachalam
Division of Genetics, Indian Agricultural Research Institute, New Delhi (India)

Summary. A set of 167 'mucromphs' was evaluated in their F_1 generation for seven 'plant', four 'plot' and five 'ratio' characters. Methods of characterizing the parents and the hybrids on the basis of combining ability effects described in the first part were found to be efficient in identifying desirable cross combinations for pedigree breeding or generating composite populations. Order effects of multiple-pollen combinations were found to be potent for a number of character components. High × Low cross-combinations were found to produce a high frequency of heterotic crosses followed by High × High. Selected biparental progenies, single crosses, three-way crosses and elite varieties were successful female parents and combinations of pollen from two varieties, one variety and three varieties were successful male parents, in that order, in producing significant heterosis in mucromphs. The relationship between general and specific combining ability on the one hand and realised heterosis on the other was discussed.

Key words: Multiple cross – Combining ability – *Brassica-campestris* – Heterosis – Multiple pollen – Oilseed breeding – Genetic classification – Mucromphs

Introduction

In part 1 of this paper (Arunachalam and Bandyopadhyay 1979) methods of studying hybrids between multiple crosses and multiple pollen (Mucromphs) were discussed. 167 such mucromphs were evaluated in their F_1 generation in a randomized blocks design in 1976 at the Indian Agricultural Research Institute, New Delhi. The material and methods have been described in complete detail in part 1 of the paper. The results obtained on the seven 'plant characters', four 'plot characters' and five 'ratio characters' described earlier, are discussed in this paper. The list of abbreviations used in this article has already been presented in Table 1 of the first part of this paper.

Results

Status of Parents Based on gca

Parents were allotted a status, high or low, on the basis of their general combining ability (gca) for 16 characters measured by an overall score, as described in part 1 of this paper. It was found (Table 1) that 50% of the female parents and only 25% of the male parents belonged to the high category. The categorization of the females would appear to be sound since the distribution of high (H) and low (L) were equal. Inter-pollen interaction might be one cause for the high proportion of male parents in the low category.

The inter-varietal single cross, KLIB was of high status. Of the two single crosses, 5995, derived from a cross between a line obtained through disruptive selection (for flowering time) of the self-incompatible KL17 (selected for earliness) and a line obtained through stabilizing selection of the self-incompatible Pusa BST-2 (selected for earliness), attained high status. The other, 1555, a cross between a line derived through disruptive selection of the self-compatible KT5907 (selected for lateness) and a line through stabilizing selection for the self-incompatible Assam Local (selected for earliness), attained low status. Two each of the triple-crosses belonged to high and low categories. The triple cross derived from an early line of the self-compatible KT5905 and I84-63 was a high parent. The other one, derived from a late line of the self-incompatible KL17 and BS182, became a low parent. Of the two triple crosses involving the intermediate-compatible GBSII, the one that underwent selection for earliness and mated to K1 was a high parent and the one that underwent selection for lateness and mated to K1 was a low

0040-5752/80/0058/0005/$ 1.20

parent. A balanced allocation of the complex and divergent triple and single crosses to high and low categories by the methods employed was thus obvious. Of the three biparental (F_2) (BIP) progenies of the inter-varietal cross, IB6 × DS17D, two were low and one was high in status. This allocation was justified since they were progenies of single plants selected from the BIP's; as such it was possible that the three lines belonged to genetically diverse groups. The F_2 progeny of the *toria* × 'brown sarson BIP', T1842 × GBSII, was a high combiner. Again out of the four BIP's, two were high and two low. Of the three varieties, the indigenous composite, 'PUKA' was high while the two exotic varieties, 'JS41' ('Japanese yellow sarson') and 'POLR' ('Canadian brown sarson') were low combiners.

To sum up, the complex female parents were justifiably grouped by the methods employed.

Of the four single pollens, 'DS17D' and 'BELE' were high combiners, the former of which was a homozygous, indigenous variety bred by disruptive selection and the latter a Swedish variety. The other Swedish variety, 'TORP' and the Indian composite, 'PUKA' were low combiners as pollen parents. But 'PUKA' (as a female parent) was a high combiner. The double-pollen combinations, DSPK and BEPK, involving 'PUKA' attained low status regardless whether the companion variety of 'PUKA' was Indian or exotic. On the other hand, BEDS, an Indian-exotic combination, was found to be high and the exotic-exotic combination TP71 a low combiner. Of the four triple-pollen combinations, all involving 'PUKA', three were low and one was high and the latter, BDP3 contained the high combination BEDS. It was thus observed that the pollen parents showed an edge towards low status.

Table 1. General combining ability status of parents and their efficiency in producing heterosis

Parents	t	s	h	hl
Females				
KLIB	2	H	3	1
5995	1	H	3	2
1555	−2	L	3	2
76I8	0	H	4	3
35KI	−2	L	0	0
73BS	−4	L	1	0
26KI	5	H	3	1
ID91	−2	L	3	3
ID92	6	H	4	1
ID21	−7	L	2	0
TG73	0	H	2	2
PUKA	2	H	2	1
JS41	−1	L	3	0
POLR	−4	L	2	1
NORM	−0.43			
Males				
BELE	5	H	1	0
TORP	−1	L	4	1
DS17	4	H	5	2
PUKA	−5	L	2	1
BEDS	7	H	6	3
DSPK	−1	L	3	2
BEPK	−5	L	1	0
TP71	−3	L	3	2
BDP3	3	H	3	1
DPI3	−1	L	2	1
TLP3	−1	L	3	2
TIP3	−2	L	2	2
NORM	0.33			

t = total score over 16 characters; s = overall status; h = number of heterotic 'mucromphs' produced by the parent; hl = number of heterotic 'mucromphs' under HL category only

The Relationship Between GCA and SCA

It was statistically tested whether the mean value of a mucromph differed significantly from that of its superior parent for each character. Such significantly superior mucromphs were only evaluated for overall heterosis by the methods described earlier (Arunachalam and Bandyopadhyay 1979). It was found that only 35 crosses were heterotic by this criterion (Table 2) and that their frequency was highest in the HL gca group, followed by HH and LL. However, the number of crosses made in each group was not constant. Further, only 20.9% of the 167 crosses were heterotic, of which a maximum number was found in the HL category. Given a heterotic cross, the probability of finding it was higher under HL than under HH or LL.

Significantly enough, all the heterotic 'mucromphs' had uniformly high sca. While a maximum proportion of het-

Table 2. Distribution of heterotic 'mucromphs' among different categories

	g	HH	HL	LL	Total
H	n	10	31	20	61
	h	7	17	8	32
HN	n	9	24	16	49
	h	2	1	0	3
HC	n	1	4	3	8
	h	0	0	0	0
Total	n	20	59	39	118
	h	9	18	8	35
L	n	8	25	16	49
	h	0	0	0	0
Total	n	28	84	55	167
	h	9	18	8	35

g = category based on gca; s = category based on sca;
n = number of crosses made; h = number of heterotic crosses

erotic crosses had high sca with scores greater than zero (Table 2), three of them were found to have zero scores due to non-significant sca for all the characters (two of which were found under HH and one under HL). The LL category recorded heterosis on the strength of high sca with scores greater than zero only.

Efficiency of Seed and Pollen Parents in Producing Heterosis

Varying efficiencies of the seed and pollen parental groups (Table 3) were recorded. For instance, single cross (SC) seed parents were successful in producing the maximum of heterotic crosses under HH while they were the least efficient under LL. On the other hand, VR, failing to produce appreciable heterosis under HH and HL proved to be otherwise under LL. DP proved to be the successful pollen combination under HH and HL and SP under LL. However, interaction of pollen and seed parents played their role in the manifestation of heterosis. For example, BP obtained the highest score as seed and DP as pollen parents under HL; but BP × DP did not produce the highest frequency of heterotic crosses. By the same analogy TC, which was only second in merit as a seed parent under HL, did produce the highest frequency of heterotic crosses when pollinated by DP. Such interactions were located in other categories as well. Based on overall performance BP, SC, TC and VR as female and DP, SP and TP as male parents were found to be potential in that order (Table 5).

A study of the frequency of heterotic crosses produced by specific parental groups revealed the relatively higher importance of eight female and three male parents in contributing to heterosis (Tables 1, 4). It was found that the crosses made between parents which produced a high frequency of heterotic crosses, were not always heterotic, implying important interaction between particular parents. Even then, it was possible to identify potential female parents like 76I8, ID92 and male parents like BEDS, DS17 and TORP for heterosis (Table 4).

Order Effects of Pollen Combinations

Of all the pollen combinations, only 'PUKA'-BELE-DS17, was examined in depth since, in this case, all possible combinations were used. It was found that the 'PUKA'-BELE effect worked significantly in the negative direction for SV, SQ, SBP, SLP and YLP including MF (which acted towards bringing lateness) (Table 5). The 'PUKA'-DS17

Table 3. Percentage of heterotic crosses produced under different categories

	HH	HL	LL	Overall
SC × SP	11.11	5.55	–	5.71
TC × SP	11.11	5.55	–	5.71
BP × SP	11.11	11.11	25.00	14.29
VR × SP	11.11	–	25.00	8.57
SP	44.44	22.22	50.00	34.29
SC × DP	11.11	11.11	–	8.57
TC × DP	11.11	16.66	12.50	14.29
BP × DP	11.11	11.11	–	8.57
VR × DP	–	5.55	12.50	5.71
DP	33.33	44.44	25.00	37.14
SC × TP	11.11	11.11	12.50	11.43
TC × TP	–	5.55	–	2.86
BP × TP	11.11	11.11	–	8.57
VR × TP	–	5.55	12.50	5.71
TP	22.22	33.33	25.00	28.57
Overall	100	100	100	100
SC	33.33	27.77	12.50	25.71
TC	22.22	27.77	12.50	22.86
BP	33.33	33.33	25.00	31.43
VR	11.11	11.11	50.00	20.00

Table 4. Order of importance of different parents in producing heterosis

	HH		HL		LL		Overall	
	P	C	P	C	P	C	P	C
Females	[a]*SC, BP*		*BP*		*VR*		*BP*	
	ID 92	3	ID 91	3	JS 41	3	ID 92	4
							ID 91	3
	KLIB	2	TG 73	2	*BP*		*SC*	
					ID 21	2	KLIB	3
			[a]*SC,TC*				5995	3
			7618	3	[a]*TC,SC*		1555	3
			26K1	2	– –		*TC*	
			5995	2			7618	4
			1555	2			26K1	3
			VR				*VR*	
			– –				JS41	3
Males	*SP*		*DP*		*SP*		*DP*	
	DS17	3	BEDS	3	TORP	3	BEDS	6
			DSPK	3				
	DP		*TP*		[a]*DP,TP*		*SP*	
	BEDS	3	TLP3	2	– –		DS17	5
							TORP	4
	TP		*SP*				*TP*	
	BDP3	2	DS17	2			––	

P = order of importance; C = number of heterotic crosses produced by the parent
[a] of the same importance; for other details, see text

Table 5. Estimated order effects of pollen combinations

Character	SV	MF	HT	PB	SB	SQ	SBP	SLP	YLP	YDS
Order effect[b]									x10⁻²	x10⁻²
I	–0.06	2.54[a]	0.15	–0.04	–1.86	–14.27	0.16	–0.10	0.16	1.02[a]
II	0.10	4.90[a]	14.55[a]	0.93	0.64	–15.46[a]	–0.09	–0.11	0.12	0.30
III	–0.18[a]	6.97[a]	5.55	0.79	–1.57	–22.75[a]	–0.29[a]	–0.21[a]	–0.83[a]	–0.01
IV	0.17	–10.35[a]	–15.24	–0.38	2.81	24.50	0.28	0.22	0.47	–0.57
V	0.03	– 7.39[a]	–16.56[a]	–0.91	–4.43[a]	–11.19	–0.14	–0.08	0.23	0.37[a]
VI	–0.01	– 1.07	2.56	0.18	0.51	0.65	–0.00	0.02	–0.04	0.31
VII	0.08	0.58	6.93	1.05[a]	4.18[a]	7.32	0.23[a]	0.08	0.09	–0.20
VIII	0.00	2.95	5.77	0.56	1.92	4.86	0.07	0.05	0.10	–0.17

[a] significant at 5% level
[b] for explanation, see part 1 of the paper, Theor. Appl. Genet. 54, (1979) p. 206

effect was significantly positive for HT and MF and negative for SQ. The BELE-DS17 effect was significant and positive for MF and YDS. However, the third-order effect, BELE-'PUKA'-DS17, was not significant for any character except MF for which it was desirable and significant. Thus it may be possible to exploit desirable pollen-order effects on quantitative characters in mucromphs.

The performance of pollen parents must, therefore, be considered in the light of the observed pollen interaction or order effects. The double-pollen combination BEDS (= BELE + DS17) was the top scorer to produce the maximum number of heterotic 'mucromphs' (Table 1). While DS17 occupied the second rank, BELE occupied the sixth and last rank. The double pollen combination BEPK (= BELE + 'PUKA') occupied the last rank while 'PUKA' occupied the fifth rank. The combination DSPK (= DS17 + 'PUKA') occupied the fourth rank. The triple pollen combination BDP3 (= BELE + 'PUKA' + DS17) also occupied the fourth rank. Thus complex interactions producing reinforcing effects (as in BEDS) or otherwise could be noticed in pollen-combinations. Of the two triple pollen-combinations, DPI3 and TIP3, in which 'PUKA' and I84-63 were common, one contained KT5907 and the other its derivative DS17, obtained fifth rank.

Discussion

The science of plant breeding has taken such rapid strides in the past few decades that old concepts are rapidly being replaced to suit the present-day requirements of a faster rate of yield improvement in crop plants. Emphasis is placed more and more on starting with a productive and diverse gene pool so that desirable and high yielding derivative-complexes can be obtained in a reasonably short time. The question naturally arises as to how a breeder can devise means of generating such a productive gene pool. As breeding experience in crops like wheat, barley, pearl millet and maize (as far back as 1939) has shown (Mangelsdorf 1939; MacKey 1965; Jensen 1970; Redden and Jensen 1974; Arunachalam and Srivastava 1980; Arunachalam and Reddy 1980), it is profitable to start with multiple in preference to single crosses. It becomes necessary then to identify ways and means of generating such multiple crosses. *Brassica campestris* offered a wide scope in being used as a test material. Multiple crosses could easily be made using complex crosses as females with mixed pollen from a number of varieties. A study of such crosses has brought out interesting results.

Characterization of Parents

The basic aim was to pool information over all the interacting characters. Of the various possibilities, a method which characterises the relative potential of a parent in a repeatable manner was preferred. The parents were categorized into two groups, one consistently far superior to a chosen norm, and the other inferior. These two situations were designated as high (H) and low (L) purely as a matter of convenience. Since the main interest was on heterosis, parents were classified based on a total score, over the characters, of tested gca effects and the crosses on sca effects. Again the choice of a norm for deciding the high and low status is an open question. The mean of the gca effects is constrained to be zero. Situations would arise where all gca effects, other than non-significant ones, are in one direction. Zero as a norm would then classify all parents in one category, high or low. But our interest was to identify even the extremes in such cases. Therefore, the mean of the significant effects was used as the norm to identify the extremes, in any given range of gca and sca effects. This approach has been found to be a viable one as the follow-up study in further generations has shown (Das 1979).

An analysis of the status of female parents revealed that, of each of the four triple crosses and biparental entries, two were high and two were low; of the three single crosses, one was low and of the three varieties, one was high; thus a balanced distribution of high and low status was obtained even between groups within females, in addition to such an overall distribution among female parents. However, the bias towards L in classifying male parents could be explained. The manner in which the pollen were applied on the stigmatic surface, would have allowed a non-uniform pollen interaction since, competition between pollen would occur only at some places on the stigma (see Part 1 of the paper) and hence the resulting seeds could exhibit preferential discrepancies in the expression of quantitative characters, for simple to complex combinations of pollen. In fact, of the four single pollens two occupied high and two low status; but only one each of the four DP and four TP was high showing a tilt of balance towards L.

But the method of categorizing a parent as high or low general combiner was found to be of high repeatability under a variety of situations. For example, 'PUKA' was of high status as female and low as male. This could be due to the possibility that the plant which was used as female was high while that used for pollen was low, as 'PUKA', a composite population, contained a number of diverse genotypes. The three single plant BIP parents, derived from a single inter-varietal cross, 'IB6' ('yellow sarson') × 'DS17D' ('brown sarson') were found to occupy different categories. The repeatability meant here was solely that of the method, and not the status of a particular parent over space, time or any other environment. Studying diallel crosses and synthetics of alfalfa for forage yield, Theurer and Ellig (1964) observed that some parents were high combiners in one spacing and low in the other, resulting in differential hybrid performance. They concluded that there was no consistent association between combining ability components and the hybrid or synthetic performance. From the concepts set forth here, this statement would need revision as, obviously, a parent could be a high combiner in one and a low combiner in another agronomic environment and could thus influence the stability of hybrid performance.

Combinations Producing Heterosis

The unique superiority of HL crosses was brought out by the study on mucromphs, and also by studies of similar nature on triticale (Arunachalam and Srivastava 1980), pearl millet (Arunachalam and Reddy 1980; Reddy and Arunachalam 1980) and barley (Fejer and Jui 1979). The concept of obtaining heterosis by mating divergent genotypes is not new and literature is abundant where the superiority of hybridizing contrasting genotypes – which we have chosen to designate as H and L – has been amply demonstrated. By hybridizing cultivated *Triticum aestivum* with the wild species *Aegilops bicornis*, highly successful variety derivatives were obtained (Riley, R. lectures delivered at I.A.R.I., New Delhi, 1976). By utilizing exotic (and temperate) × Indian (and tropical) and dwarf × tall crosses, several hybrids and varieties were evolved in sorghum (Rao 1972; Rao and Rana 1978). The mating of lines with contrasting attributes generated by disruptive and stabilizing selection produced potential derivatives in 'brown sarson'. From three-way crosses like (original × productive recombinants) × productive cultures, high yielding populations could be obtained in 'brown sarson' (Arunachalam and Katiyar 1978). The high-low method of breeding was described in excellent detail by Langham (1961).

On the Relationship Between GCA, SCA and Heterosis

Theoretical analysis (Arunachalam 1977) of the components of heterosis in two-gene system indicated a possibility of realizing heterosis on the strength of additive effects and additive type of interaction only. This was borne out by this study too, where about 10% of the heterotic mucromphs were found to have non-significant sca effect for every character. Two of these crosses were found under HH and one under HL categories. None of these crosses could be found under LL, as reinforcing additive × additive interactions would not alone be expected to produce heterosis under LL. But heterotic mucromphs with non-significant sca for every character would be preferred for population breeding as it would be possible to sustain their F_1 superiority over the generations.

Thus a knowledge of sca alone would not be adequate to form an idea of the necessary conditions for obtaining heterosis. As such, the hypothesis of heterosis stressing the role of dominance and dominance-based interactions needs revision. Studies relating sca effects with hybrid performance without caring for the gca of the parents confirmed the above observation. For example, a one-to-one relationship between the magnitude of sca effect and hybrid yield (Singhania 1972; Singh and Jain 1971) was observed in some cases and no relationship at all (see, for example, Singh and Singh 1974) in some others. The ambiguous conclusions arrived at by considering the parental combinations as HH, HL etc., on the basis of gca for one chosen component character, discussing the presence or absence of heterosis and relating it further to the sca components (Gill et al. 1974) would only confirm the superiority of the methods and the results of our study.

Would, then, the established superiority of H × L crosses, in producing heterosis, be of practical utility to a breeder? This question can have, partly at least, an unambiguous answer. It may not be possible to predict the percentage of crosses that would prove to be heterotic, though this would normally be expected to be small. For instance, only 35 out of the 167 mucromphs proved to be heterotic in our study. But given a heterotic cross, the probability of finding it to be a H × L combination was high (in our study, 18 of the 35 heterotic crosses, were found under HL, Table 2). So when a breeder has to attempt successful hybridization economically (in terms of time, cost and number of crosses), it is worthwhile to start with the H × L type of crosses followed by H × H for realizing heterosis. This statement is in perfect agreement with the well established concept of making crosses between divergent genotypes (in terms of geographic origin, genetic potential etc.). This was further borne out by a casual phenotypic assessment of the F_1 mucromphs in the field, three weeks before harvest. Of the 40 crosses, showing productive phenotypic appearance, 3, 26 and 11 belonged respectively to the HH, HL and LL categories, which was in tune with the observations made earlier. However, the distribution of the groups like SC, DP etc., in the productive crosses did not exactly follow the pattern arrived at earlier. This would only emphasize the inadequacies of visual assessment, when a large number of mucromphs with varying potential were simultaneously growing in the field with obvious differences in their growth attributes.

The results discussed so far lead us to the next important topic: how can one organize an investigation with a view to identifying a strategy for increasing the rate of yield improvement? What characters should be considered and with what weightage? and finally the strategy itself. These will be considered in depth in the next and concluding part of this paper.

Literature

Arunachalam, V. (1977): Heterosis for characters governed by two genes. J. Genet. **63**, 15-24

Arunachalam, V.; Bandyopadhyay, A. (1979): Are 'Multiple cross-multiple pollen hybrids' an answer for productive populations in *Brassica campestris* var. 'brown sarson'? 1: Methods for studying 'Mucromphs'. Theor. Appl. Genet. **54**, 203-207

Arunachalam, V.; Katiyar, R.K. (1978): Genetic diversity and breeding potential from disruptive selection in *Brassica campestris* cv 'brown sarson'. Expl. Agric. **14**, 1-7

Arunachalam, V.; Reddy, B.B. (1980, in press): Evaluation of heterosis through combining ability in pearl millet. 2. Multiple crosses. Indian J. Genet.

Arunachalam, V.; Srivastava, P.S.L. (1980): Assessment of genetic potential of multiple crosses in triticale. Genet. Agr. **35**, 117-127

Das, G.R. (1979): Short-term strategies of population breeding from multiple crosses in rapeseed (*Brassica campestris* L.). Ph.D. thesis, P.G. School, IARI., New Delhi

Fejer, S.O.; Jui, P.Y. (1979): Genotype-environment interactions in single seed propagated barley diallel. Cereal Res. Comm. **7**, 85-91

Gill, K.S.; Dhillon, S.S.; Arora, B.S. (1974): Line × Tester analysis of combining ability and heterosis in wheat. Breeding researches in Asia and Oceania. Indian J. Genet. **34A**, 601-609

Jensen, N.F. (1970): A diallel selective mating system for cereal breeding. Crop Sci. **10**, 629-635

Langham, D.G. (1961): The High-low method in crop improvement. Crop Sci. **1**, 376-378

MacKey, J. (1965): Combination procedures in wheat and barley breeding. FAO Bulletin **16**, 13-17

Mangelsdorf, P.C. (1939): Use of multiple top-crosses in southern corn improvement. Mimeo. Rept. First Southern Corn Improvement Conf. **1**, 33-35

Rao, N.G.P. (1972): Sorghum breeding in India: recent developments. in: Sorghum in seventies. (eds.: Rao, N.G.P.; House, L.R.), pp. 101-142. New Delhi, Bombay, Calcutta: Oxford and IBH.

Rao, N.G.P.; Rana, B.S. (1978): Characterization of tropical and temperate sorghums and their utilization. Proc. Nat. Symp. Plant and Animal Genetic Resources, New Delhi. 28-30

Redden, R.J.; Jensen, N.F. (1974): Mass selection and mating system in cereals. Crop Sci. **14**, 345-350

Reddy, B.B.; Arunchalam, V. (1980, in press): Evaluation of heterosis through combining ability in pearl millet. I. Single crosses. Indian J. Genet.

Singh, K.B.; Jain, R.P. (1971): Analysis of diallel cross in *Phaseolus aureus* Roxb. Theor. Appl. Genet. **41**, 279-281

Singh, T.P.; Singh, K.B. (1974): Heterosis and combining ability in *Phaseolus aureus* Roxb. Theor. Appl. Genet. **44**, 12-16

Singhania, D.L. (1972): Heterosis, line per se performance, combining ability and stability of performance in grain sorghum (*Sorghum vulgare* bicolor (L) Moench.). Ph.D. Thesis, P.G. School, IARI., New Delhi

Theurer, J.C.; Ellig, L.J. (1964): Comparative performance of diallel crosses and related second generation synthetics of alfalfa, *Medicago sativa* L. 3: Forage yield. Crop Sci. **4**, 25-28

Received February 4, 1980
Communicated by B.R. Murty

Dr. A. Bandyopadhyay
Dr. V. Arunachalam
National Research Centre,
IARI-Regional Station,
Rajendranagar,
Hyderabad 500 030 (India)