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Combining ability and heterosis studies for oil and seed meal quality traits in Indian mustard (*Brassica juncea* L.)

Priyamedha*, Arun Kumar, CS Mahto and ZA Haider

Department of Plant Breeding and Genetics, BAU, Ranchi-834 006, Jharkhand, India
** Corresponding author: priyamedha.pb@gmail.com*
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Abstarct

The study of combining ability, and heterosis for oil and seed meal quality traits in 40 F_1s of Indian mustard, developed from 8 lines, and 5 testers was carried out in randomized block design with two replications at Crop Research Centre of Birsa Agricultural University, Ranchi, India during *Rabi* 2014-15. Analysis of variance revealed, significant differences among genotypes for all the traits. Higher gca variance indicated the role of additive gene action in inheritance of these traits except saturated fatty acid, and linolenic acid. The parents, Shivani and Heera were good combiner for most of the quality traits. Estimation of specific combining ability effects of crosses *viz*., NRCHB 101 x Heera, Pusa Bold x Pusa Mustard-21, Shivani x Heera, Pusa Mustard-25 x RGN-73, and BAUSM 92-1-1 x Pusa Mustard-21 were highly significant for glucosinolate content, erucic acid, oleic acid, and linoleic acid. Considering mean performance, and general combining ability effect, the parent, Heera was found promising for glucosinolate content, linoleic acid, and erucic acid. On the basis of mean performance, specific combining ability effect, and heterobeltiosis, the crosses, BAUSM 92-1-1x Pusa Mustard-21 for linoleic acid, and erucic acid, Shivani x BPR 543-2 for saturated fatty acid, NRCDR-02 x RGN-73 for linolenic acid, and NRCDR-02 x Heera for glucosinolate content were found promising. The cross, Shivani x Heera was found to be most promising showing desirability for maximum number of quality traits. Selection in early segregating generation is suggested to be more effective for breeding for quality traits in Indian mustard as most of them were found to be controlled by additive genes action.

Keywords: *Combining ability, heterosis, Indian mustard, quality traits*

Introduction

Indian mustard [*Brassica juncea* (L.) Czern & Coss.] is an agriculturally important oilseed crop with a long history of cultivation in India, accounting for about 75-80% of total area, and production under rapeseed-mustard cultivation. It contributes nearly 30% of the total oilseeds, and 27% to edible oil pool of the country. Indian mustard seed contains 35-44% oil. Mustard oil is an important dietary component in India, especially in Eastern, and North-Western parts.

The nutritional quality, and shelf life of oil is determined by its fatty acid composition. The mustard oil contains <7% saturated fatty acid (palmitic and stearic acid). It is also a rich source of unsaturated fatty acids *viz.,* oleic acid (C 18:1), linoleic acid (C 18:2), linolenic acid (C 18:3), and erucic acid (C 22:1). Mustard oil is considered suitable for use as edible oil due to presence of higher proportion of monounsaturated fatty acids (C 16 and C 18), and less amount of saturated fatty acid (Supriya *et al.* 2014). However, it contains high amount of erucic acid (40-50%), which is considered nutritionally undesirable (Singh *et al*., 2014). In addition, feed value of seed meal remaining as a by-product after extraction of oil is lost by the presence of high amount of glucosinolate (80-160 µmoles /g defatted seed meal) (Wanasundara, 2011). It is a large group of secondary plant metabolites derived from different amino acids such as methionine, phenylalanine, and tryptophan. Therefore, extensive breeding efforts are in progress for development of low erucic acid (<2%), and low glucosinolate (<30 µmoles /g defatted seed meal) genotypes in Indian mustard (Priyamedha *et al.,* 2015).

The combining ability analysis gives an idea about the relative magnitude of additive, and non-additive types of gene action in expression of the traits in varietal improvement program. Combining ability studies provide useful information for selection of good combiners, which are expected to give high performance in their crosses, and progenies. The knowledge of combining ability is also useful to get information on nature of gene actions involved in the inheritance of traits under study. General combining ability is primarily a function of additive gene action and additive x additive interaction, whereas, specific combining ability is due to non-allelic gene interaction. Nature, and magnitude of combining ability effects help in identifying superior parents, and their utilization in further breeding programme. Study on heterosis is useful in deciding the direction, and prospects of future improvement programme. It is also important to find out the specific improvement programme, which might be more promising than the conventional breeding programme. The present study was made with a view to study the combining ability, and heterosis of Indian mustard genotypes, and their crosses to identify good combiner genotypes for best cross combinations, which can be utilized for selection in succeeding segregating generations for oil, and seed meal quality traits.

Materials and Methods

Plant Materials

The material for present study consisted of 53 genotypes of Indian mustard having 8 lines (NRCHB 101, NRCDR-02, Kranti, Pusa Bold, Shivani, Pusa Mustard- 25, BAUSM-92-1-1 and BAUM 2007), 5 testers (Pusa Mustard- 21, Heera, JN032, RGN-73, and BPR 543-2), and their 40 crosses developed in line x tester design. The crosses along with parents were evaluated during *rabi* 2014-15 at Crop Research Centre of Birsa Agricultural University, Ranchi, India in randomized block design with 2 replications. Each genotype was sown in single row of 3 m length spaced 30 cm apart with plant to plant spacing of 10 cm achieved by thinning after 15-20 days of sowing. Recommended package of practices for raising a healthy crop was followed. Five plants were randomly selected from each genotype in each replication to record data on oil content (%), glucosinolate content (µmoles/g of oil-free seed meal), saturated fatty acid (%), oleic acid (%), linoleic acid (%), linolenic acid (%), and erucic acid (%).

Determination of oil content and seed meal preparation

The seeds were thoroughly ground in a pestle and mortar, and 10.0 g triplicates of ground seeds were extracted with hexane for 24 h in a Soxhlet apparatus (AOAC, 1997). Subsequently, hexane was removed from the oil by rotary evaporator under reduced pressure, and the weights of the residual oils were calculated. The seed meal remaining after the extraction of total oil was preserved for estimation of glucosinolates.

Estimation of glucosinolates

Total glucosinolate content in the seed meal was estimated by complex formation between glucosinolates, and sodium tetrachloropalladate solution. The intensity of the color produced was measured using spectrophotometer at 405 nm (Kumar *et al*., 2004).

Fatty acid analysis by Gas Liquid Chromatography (GLC)

Methyl esters of oil samples were prepared by transesterification according to the method described by Sarin *et al*. (2009) with slight modifications. 1.0 ìl of the methyl ester sample was injected into SP 2300 + 2310 SS column. A Nucon model 5765 gas chromatograph equipped with flame ionization detector (FID) was used. The oven, injector, and detector temperature were 240 °C, 230 °C, and 250 °C, respectively. The carrier gas was nitrogen, at flow rate of 40-50 ml/min. Peaks of fatty acid methyl esters were identified by comparing their retention time with that of the known standards run under similar separation conditions. Individual fatty acids were expressed as % of the total fatty acids.

Statistical analysis

Mean data were subjected to analysis of variance (ANOVA) as suggested by Panse and Sukhatme (1978). The combining ability analysis of parents, and crosses was taken up for different characters using the line x tester model as given by Kempthorne (1957). Heterobeltiosis was calculated as method given by Matzingar *et al.* (1962).

Results and Discussion

The analysis of variance revealed highly significant mean square values for genotypes, parents, hybrids, and parents vs. hybrids indicating sufficient genetic variability in the material for all the characters under study (Table1). Similar pattern have also been observed by Hu *et al.* (1996), and Turi *et al.* (2010). Comparison of mean squares due to parents vs. hybrids were found highly significant for all the characters under study except for oil, and glucosinolate content, indicating, hybrids differ significantly from that of the parents for these traits by presence of mean heterosis for all these characters. The analysis of variance for combining ability revealed that the mean squares due to testers were significant for all the characters studied except linolenic acid. This indicated significant contribution of testers towards gca variance component for these traits (Table 2). The mean squares due to tester were greater than those due to line for all the characters studied, which indicate larger diversity among the

Table 1: ANOVA showing mean sum of squares of parents, and crosses for quality traits in Indian mustard

Sources	D.F.	Oil content (%)	Glucosinolate (µM/g of oil-free seed meal)	Saturated fatty acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Erucic acid (%)
Replicates	1	0.01	0.05	0.05	0.10	0.13	0.00	0.04
Parents	12	1.31**	791.35**	0.35**	111.72**	26.52**	6.28**	455.19**
Line	7	0.54*	265.19**	0.18**	2.26**	2.74**	1.99**	50.73**
Tester	4	2.58**	1004.85**	0.68**	186.36**	44.24**	14.75**	703.29**
Line vsTester	1	1.65**	3620.41**	0.14**	579.43**	122.13**	2.42**	2293.94**
Parents vs Crosses	1	0.00	0.20	0.14**	24.32**	8.50**	6.45**	4.48**
Error	52	0.19	0.25	0.01	0.04	0.04	0.05	0.07

* and ** Significant at P = 0.05 and P = 0.01, respectively.

Table 2: ANOVA for combining ability, and estimates of components of variance for quality traits in Indian mustard

Sources	D.F.	Oil content (%)	Glucosinolate (µM/g of oil-free seed meal)	Saturated fatty acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Erucic acid (%)
Replicates	1	0.01	0.05	0.05	0.10	0.13	0.00	0.04
Crosses	39	0.82**	797.43**	0.52**	19.02**	16.42**	4.54**	137.56**
Line effect	7	0.85	214.63	0.22	4.85	6.45	4.19	75.27
Tester effect	4	3.66**	4894.68**	1.81**	109.21**	79.34**	6.88	952.90**
L x T effect	28	0.40*	357.81**	0.42**	9.68**	9.92**	4.29**	36.65**
Error	52	0.19	0.25	0.01	0.04	0.04	0.05	0.07
Components of variances								
σ^2 a (2 * σ^2 GCA)		0.32	392.98	0.15	8.77	6.59	0.84	79.08
σ^2 D (σ^2 SCA)		0.11	178.78	0.20	4.82	4.94	2.12	18.29
σ^2 a / σ^2 D		2.97	2.20	0.77	1.82	1.33	0.40	4.32

* and ** Significant at P = 0.05, and P = 0.01, respectively.

testers than in line for these characters. The variance due to gca was higher than that of due to sca for all the characters except saturated fatty acid, and linolenic acid. This indicated the role of additive gene action in the inheritance of these traits. Similar results were concluded by Turi *et al*. (2010), and Patel *et al.* (2015).

An overall appraisal of general combining ability effects of parents revealed that none of the parents was found to be a good general combiner for all the characters (Table 3). However the parent, Shivani was good combiner for oil content, saturated fatty acid, oleic acid, linoleic acid and linolenic acid. Likewise, Heera was good general combiner for glucosinolate content, oleic acid, linoleic acid, linolenic acid, and erucic acid. Among the parents, Pusa Bold, Kranti, JN032, BPR 543-2, Pusa Mustard-21 and Pusa Mustard-25 were good general combiner for more than two characters. The parent, Heera was found good general combiner with lowest mean for glucosinolate content, and erucic acid as well as highest mean for linoleic acid. Likewise parents, Pusa Mustard-21, and RGN-73 showed good combining ability with highest content of oleic acid, and lowest content of linolenic acid respectively. The significant general combining ability effects are responsible for additive or additive x additive gene effects for the inheritance of that particular trait (Spragme, 1966). In the present study, all the quality traits had significant GCA effects which revealed that they are of fixable in nature, and by adopting simple selection these traits can be improved in Indian mustard. The parents which are good general combiners for these traits could be used in further crossing programme.

Estimation of specific combining ability effects of crosses revealed that out of 40 crosses, none of the cross showed consistently high SCA effect for all the characters under study. The crosses, NRCHB-101 x Heera, NRCDR-02 x Heera, NRCDR-02 x JN-032, Shivani x Heera, Shivani x BPR 543-2, Pusa M-25 x RGN-73, BAUSM92-1-1 x Pusa M-21, and BAUM-2007 x RGN-73 showed highly significant negative sca effect for glucosinolate content, and erucic acid. Whereas the crosses, NRCHB-101 x Heera, Pusa Bold x Pusa M-21, Shivani x Heera, Kranti x RGN-73, BAUSM92-1-1 x Pusa M-21, BAUSM92-1-1 x RGN-73, BAUM-2007 x Heera, and BAUM-2007 x BPR 543-2 showed highly significant positive sca effect for both oleic acid, and linoleic acid. The cross, BAUSM92-1-1 x JN032 showed highly significant negative sca effect for

Table 3: Significant GCA effects, and mean performance of parents for seven quality traits

Characters	Parents
Oil Content (%)	Shivani (0.48**, 41.97), BPR 543-2 (0.60**, 41.33)
Glucosinolate (µM/g of oil-free seed meal)	Pusa Bold (-5.93**, 77.14), Kranti (-6.29**, 64.17), BAUM-2007 (-3.26**, 78.96), Heera (-25.08**, 26.38), JN032 (-10.09**, 38.61)
Saturated fatty acid (%)	NRCHB 101 (-0.12**, 3.05), Pusa Bold (-0.23**, 3.66), Shivani (-0.15**, 3.39), RGN-73 (-0.18**, 3.19), BPR 543-2 (-0.30**, 3.44), JN032 (0.24**, 3.30)
Oleic acid (%)	Shivani (0.98**, 10.48), Pusa Mustard-25 (0.98**, 10.63), BAUSM-92-1-1 (0.21**, 12.74), Pusa Mustard-21 (3.91**, 30.43), Heera (1.34**, 29.77)
Linoleic acid (%)	NRCHB 101 (0.28**, 18.66), Shivani (1.00**, 19.55), Pusa Mustard-25 (0.33**, 16.85), BAUM-2007 (1.04**, 19.49), Pusa Mustard-21 (2.34**, 24.13), Heera (2.53**, 28.14)
Linolenic acid (%)	NRCDR-02 (-0.73**, 14.74), Pusa Bold (-0.77**, 14.50), Shivani (-0.23**, 16.24), Heera (-0.16**, 19.78), RGN-73 (-0.52**, 13.26), BPR 543-2 (-0.46**, 13.59)
Erucic acid (%)	Pusa Mustard-25 (-2.57**, 38.53), Kranti (-0.83**, 35.27), BAUSM-92-1-1 (-5.18**, 24.21), Pusa Mustard-21 (-7.72**, 2.12), Heera (-6.93** 1.77), JN032 (-1.34**, 3.71)

** Significant at P = 0.01.

Table 4: Crosses with highly significant SCA effects along with mean performance, and heterobeltiosis for seven quality traits

Characters	Crosses
Oil Content (%)	NRCDR-02 x BPR 543-2 (0.74, 42.45, 0.44), Kranti x JN-032 (0.72, 42.26, -0.31)
Glucosinolate (µM/g of oil-free seed meal)	NRCHB-101 x Pusa M-21 (-5.05, 84.61, -4.56**), NRCHB-101 x Heera (-12.65, 35.49, -59.97**), NRCHB-101 x JN-032 (-10.87, 52.27, -41.04**), NRCDR-02 x Heera (-13.18, 33.56, -66.93**), NRCDR-02 x RGN-73 (-5.79, 70.54, -30.48**), NRCDR-02 x JN-032 (-20.97, 40.76, -59.83**), Pusa Bold x RGN-73 (4.84, 65.14, -15.56**), Pusa Bold x BPR 543-2 (-2.38, 77.32, 0.24) , Shivani x Pusa M-21 (-1.91, 89.50, 17.20**), Shivani x Heera (-12.37, 37.52, -50.87**), Shivani x BPR 543-2 (-6.78, 82.42, 7.93**), Pusa M-25 x Pusa M-21 (-6.52, 85.15, -3.07**), Pusa M-25 x RGN-73 (-15.81, 63.93, -27.23**), Pusa M-25 x BPR 543-2 (-12.88, 76.58, -12.83**), Kranti x BPR 543-2 (-14.66, 64.69, -10.33**), Kranti x JN-032 (-7.01, 48.02, -25.18**), BAUSM92-1-1 x Pusa M-21 (-11.39, 82.29, 11.77**), BAUSM-92-1-1 x BPR 543-2 (-3.31, 88.16, 22.21**), BAUM-2007 x RGN-73 (-7.58, 65.07, -17.59**), BAUM-2007 x BPR 543-2 (-8.75, 73.63, -6.76**)
Saturated fatty acid (%)	NRCHB-101 x PUSA M-21 (-0.61, 3.08, -32.97**), NRCHB-101 x RGN-73 (-0.30, 2.78, -12.72**), Pusa Bold x Heera (-0.51, 2.95, -19.43**), Shivani x Pusa M-21 (-0.26, 3.40, -26.01**), Shivani x BPR 543-2 (-1.03, 1.91, -44.54**), Pusa M-25 x RGN-73 (-0.44, 2.85, -22.16**), BAUSM92-1-1 x JN-032 (-0.66, 2.64, -20.03**), BAUM-2007 x Heera (-0.50, 3.23, -16.75**)
Oleic acid (%)	NRCHB-101 x Heera (3.00, 17.31, 41.87**), NRCDR-02 x JN-032 (3.92, 16.55, -21.97**), Pusa Bold x Pusa M-21 (2.42, 19.67, -35.35**), Shivani x Heera (1.72, 17.55, -41.05**), Shivani x JN-032 (0.96, 14.53, -31.49**), Pusa M-25 x RGN-73 (1.86, 14.28, 34.34**), Kranti x RGN-73 (1.39, 12.12, 14.83**), Kranti x BPR 543-2 (1.50, 12.07, 9.03**), BAUSM92-1-1 x Pusa M-21 (4.45, 22.09, -27.40**), BAUSM92-1-1 x RGN-73 (1.76, 13.41, 5.26**), BAUSM92-1-1 x BPR 543-2 (0.89, 12.38, -2.83), BAUM-2007 x Heera (1.39, 15.48, -48.00**), BAUM-2007 x BPR 543-2 (1.16, 11.68, -6.26**), BAUM-2007 x JN-032 (0.53, 12.36, -41.73**)
Linoleic acid (%)	NRCHB-101 x Heera (0.80, 24.28, -13.72**), NRCHB-101 x RGN-73 (1.91, 21.15, 13.35**), NRCHB-101 x JN-032 (1.77, 21.39, -16.98**), NRCDR-02 x Pusa M-21 (0.99, 22.83, -5.37**), NRCDR-02 x Heera (0.55, 22.58, -19.76**), NRCDR-02 x BPR 543-2 (1.85, 19.55, 5.56**), Pusa Bold x Pusa M-21 (2.88, 25.38, 5.20**), Pusa Bold x JN-032 (0.62, 19.45, -24.51**), Shivani x Heera (1.15, 25.35, -9.93**), Shivani x BPR 543-2 (1.25, 21.11, 7.95**), Pusa M-25 x JN-032 (0.55, 20.21, -21.56**), Kranti x RGN-73 (1.09, 19.84, 14.72**), BAUSM92-1-1 x Pusa M-21 (5.29, 27.52, 14.07**), BAUSM92-1-1 x RGN-73 (1.68, 19.86, 0.97), BAUM-2007 x Heera (3.26, 27.50, -2.27**), BAUM-2007 x BPR 543-2 (1.23, 21.13, 8.41**)
Linolenic acid (%)	NRCHB-101 x Pusa M-21 (-1.86, 16.36, 1.39), NRCHB-101 x Heera (-0.70, 16.26, -17.77**), NRCHB-101 x BPR 543-2 (-1.18, 15.48, -4.06**), NRCDR-02 x RGN-73 (-2.68, 12.14, -17.67**), Pusa Bold x BPR 543-2 (-1.03, 13.82, -4.69**), Shivani x Heera (-0.84, 14.83, -25.01**), Shivani x RGN-73 (1.58, 13.73, -15.46**), Pusa

	M-25x Heera (-1.26, 14.60, -26.19**), Pusa M-25 x JN-032 (-1.42, 14.63, -6.13**), Kranti x RGN-73 (-0.84, 14.74, -16.68**), Kranti x JN-032 (-0.50, 15.63, -11.65**), BAUSM92-1-1 x Pusa M-21 (-0.83, 16.23, 5.80**), BAUSM92-1-1 x Heera (-0.95, 14.85, -24.91**), BAUSM92-1-1 x JN-032 (-1.13, 14.87, -3.06*), BAUM-2007 x BPR 543-2 (-1.14, 15.26, -2.43), BAUM-2007 x JN-032 (-1.47, 15.43, -1.37)
Erucic acid (%)	NRCHB-101 x Heera (-4.58, 16.54, -55.56**), NRCHB-101 x RGN-73 (-1.74, 33.55, -9.84**), NRCDR-02 x Heera (-1.77, 21.54, -47.79**), NRCDR-02 x BPR 543-2 (-2.60, 36.37, -11.85**), NRCDR-02 x JN-032 (-2.35, 26.54, -35.68**), Pusa Bold x Pusa M-21 (-6.90, 15.74, -58.03**), Pusa Bold x JN-032 (-4.52, 24.49, -34.70**), Shivani x Heera (-0.55, 21.70, -39.06**), Shivani x BPR 543-2 (-3.27, 34.65, -4.64**), Pusa M-25 x Pusa M-21 (-0.68, 16.65, -56.79**), Pusa M-25 x RGN-73 (-6.02, 26.28, -31.79**), Kranti x RGN-73 (0.61, 33.43, -10.01**), Kranti x BPR 543-2 (-3.75, 31.78, -12.52**), Kranti x JN-032 (-1.60, 23.85, -32.37**), BAUSM92-1-1 x Pusa M-21 (-5.07, 9.65, -60.16**), BAUSM92-1-1 x Heera (-1.46, 14.05, -41.97**), BAUM-2007 x Heera (6.41, 15.55, -55.09**), BAUM-2007 x RGN-73 (-3.84, 32.30, -13.07**)

** Significant at P = 0.01.

saturated fatty acid, and linolenic acid. Similar results have also been reported in some segregants of Indian mustard by Bhatt *et al*. (2008), and Patel *et al.* (2015).

The crosses, viz. NRCHB-101 x Heera (-59.97%), NRCDR-02 x Heera (-66.93%), NRCDR-02 x JN-032 (-59.83%), Shivani x Heera (-50.87%) exhibited >50% heterobeltiosis, highly significant sca effects, and lower *per se* performance for glucosinolate content. The crosses like, NRCHB-101 x Pusa M-21 (-32.97%), Pusa Bold x Heera (-19.43%), Shivani x BPR 543-2 (-44.54%), Pusa M-25 x RGN-73 (-22.16%), BAUSM92-1-1 x JN032 (-20.03%), and BAUM 2007 x Heera (-16.75%) exhibited >15% heterobeltiosis, highly significant sca effects, and lower *per se* performance for saturated fatty acid content in oil. The crosses *viz*., NRCHB-101 x Heera (41.87%), and Pusa M-25 x RGN-73 (34.34%) exhibited >30% heterobeltiosis, highly significant sca effects and higher *per se* performance for oleic acid content in oil. Likewise, crosses *viz.* NRCHB-101 x RGN-73 (13.35%), and BAUSM92-1-1 x Pusa M-21 (14.07%) exhibited >10% heterobeltiosis, highly significant sca effects, and higher *per se* performance for linoleic acid content in oil. The crosses like, NRCHB-101 x Heera (-17.77%), NRCDR-02 x RGN-73 (-17.67%), Shivani x Heera (-25.01%), Pusa M-25 x Heera (-26.19%), BAUSM92-1-1 x Heera (-24.91%) exhibited >15% heterobeltiosis, highly significant sca effects, and lower *per se* performance for linolenic acid content in oil. Similarly the crosses, viz. NRCHB-101 x Heera (-55.56%), NRCDR-02 x Heera (-47.79%), NRCDR-02 x JN-032 (35.68%), Pusa Bold x Pusa M-21 (-58.03%), Shivani x Heera (-39.06%), Pusa M-25 x Pusa M-21 (-56.79%), BAUSM92-1-1 x Pusa M-21 (-60.16%), BAUSM92-1-1 x Heera (-41.97%) and BAUM-2007 x Heera (-55.09%) exhibited >35% heterobeltiosis, highly significant sca effects, and lower *per se* performance for erucic acid content in oil (table 4). Hu *et al*. (1996), Ali *et al.* (2015) and Patel *et al*. (2015) have also reported heterobeltiosis in desired directions for these quality characters.

These crosses can be advanced further through selfing, and can be used in crop improvement programme for quality traits. Selection in early segregating generation is suggested to be more effective for breeding for quality traits as most of them were found to be controlled by additive genes action.

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