

Deciphering the genetic basis of heterosis for yield and its component traits using *Brassica carinata*-derived *B. juncea* alien introgression lines

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Electronic Supplementary Material (ESM)

The authors are fully responsible for both the content and the formal aspects of the electronic supplementary material. No editorial adjustments were made.

2 Table S1. Analysis of variance for seed yield and its contributing traits in a set of hybrids generated between *B. carinata* derived *B. juncea* introgression lines (ILs) of two different genetic backgrounds and their respective recipient parents evaluated along with parents in randomised complete block design (RCBD) design

Source of variation	Experiment a [#]				Experiment b ^{\$}			
	replication	treatment	error	CV (%)	replication	treatment	error	CV (%)
Degrees of freedom	2	54	108		2	26	52	
		mean sum of squares				mean sum of squares		
Plant height (cm)	89.33	443.94**	93.87	4.75	8.81	221.36**	71.56	3.99
Point to first branch (cm)	147.07	324.28**	52.76	13.17	45.82	289.33**	31.31	9.33
Primary branches/plant	0.18	0.86**	0.2	10.23	1.2**	1.32**	0.2	9.5
Secondary branches/plant	20.07**	9.31**	2.52	13.97	9.06*	14.77**	2.07	11.94
Main shoot length (cm)	47.98	141.73**	26.04	6.04	5.16	146.37**	20.06	5.12
Point to first silique on main shoot (cm)	2.02	9.2**	1.41	13.16	1.12	3.05**	0.71	10.78
Silique length (cm)	0.16	0.63**	0.06	6.44	0.55**	0.60**	0.09	7.57
Seeds/silique	2.83*	6.02**	0.74	6.43	0.25	3.04**	0.56	5.67
Total silique on main shoot	187.78**	52.63**	15.77	7.99	1.25	85.72**	11.18	6.64
Total number of silique/plant	7600.48*	5197.65**	1806.8	13.72	7570.36*	8479.64**	1754.37	12.28
Seed yield per plant (g)	59.54**	45.3**	6.61	13.55	47.47**	46.62**	6.66	13.27
Biological yield per plant (g)	1289.63**	552.99**	112.57	13	815.53**	647.55**	100.93	12.25
Oil content (%)	28.18**	13.38**	1.3	3.19	28.75**	6.19**	0.91	2.73
Days to 50% flowering	15.44**	35.94**	2.79	3.5	0.16	9.58**	1.28	2.38
1 000-seed weight (g)	0.1	0.73**	0.14	8.06	0.07	1.58**	0.17	8.1
Harvest index (%)	9.73	22.34**	7.66	11.86	12.78	11.34**	5.26	9.67
Seed yield/ha (t)	0.1	1.37**	0.09	11.91	0.02	0.58**	0.08	11.33

**, Significant at $P = 0.05, 0.01$; CV – coefficient of variation

– includes hybrids (DRMRIJ31_ILHs) generated between ILs of DRMRIJ 31 (DRMRIJ31_ILs) and DRMRIJ 31 along with parents; \$ – includes hybrids (PM30_ILHs) generated between ILs of Pusa Mustard 30 (PM30_ILs) and Pusa Mustard 30 along with parents

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Table S2. Performance of introgression line hybrids (ILHs) generated in DRMRJ 31 genetic background in comparison to mid-parent values, respective introgression lines (ILs), and DRMRJ 31 for yield contributing traits

Traits	Heterosis over mid-parental value		Heterosis over respective ILs		Heterosis over DRMRJ-31	
	mean	range	mean	range	mean	range
Plant height	0.19	-10.35 – 7.13	3.28	-10.05 – 12.58	-2.52	-10.80 – 6.75
Point to first branch	8.35	-11.11 – 25.06	13.86	-23.19 – 48.43	6.04	-15.69 – 23.41
Primary branches/plant	-3.57	-31.83 – 17.16	-8.84	-44.21 – 18.65	3.15	-12.40 – 19.01
Secondary branches/plant	4.50	-13.60 – 32.65	-3.76	-34.66 – 46.9	16.48	-2.82 – 42.25
Main shoot length	2.36	-5.60 – 17.75	3.65	-9.11 – 43.99	1.73	-11.81 – 11.57
Point to first siliqua on main shoot	3.00	-20.00 – 25.12	0.55	-40.48 – 58.75	8.25	-13.82 – 32.52
Siliqua length	6.65	-9.32 – 23.38	10.61	-14.10 – 45.88	3.86	-15.52 – 22.41
Seeds/siliqua	0.20	-11.47 – 16.37	12.70	-6.05 – 38.98	-9.29	-18.55 – 3.84
Total siliquae on the main shoot	-2.26	-11.20 – 8.04	6.42	-4.88 – 23.54	-9.30	-20.50 – 0.71
Total number of siliquae/plant	5.16	-17.67 – 33.98	4.16	-20.96 – 56.04	7.47	-18.37 – 33.01
Seed yield/plant	21.92	-8.03 – 57.45	35.91	-10.16 – 103.41	12.57	-12.04 – 38.92
Biological yield/plant	15.80	-13.96 – 50.09	16.23	-17.51 – 57.45	16.67	-13.04 – 43.39
Oil content	0.37	-11.97 – 6.58	3.16	-10.34 – 12.76	-2.06	-13.55 – 6.37
Days to 50% flowering	-6.22	-13.25 – 4.17	-4.52	-20.44 – 9.49	-7.55	-12.58 – -0.66
1 000-seed weight	13.81	5.71 – 26.09	8.98	-6.33 – 34.26	20.00	5.74 – 38.52
Harvest index	5.03	-21.23 – 24.27	15.60	-20.71 – 51.43	-3.11	-21.74 – 11.72
Seed yield/ha (t)	29.70	-7.01 – 73.05	60.17	-2.67 – 161.78	12.59	-12.19 – 48.78

4 Table S3. Performance of introgression line hybrids (ILHs) generated in Pusa Mustard 30 genetic background over mid-parent value, respective introgression lines (ILs), and Pusa Mustard 30 for yield contributing traits

Traits	Heterosis over mid-parental value			Heterosis over respective ILs			Heterosis over Pusa Mustard 30		
	mean	range		mean	range		mean	range	
Plant height	1.95	-3.45 – 7.21		3.32	-2.12 – 13.64		0.72	-4.75 – 5.40	
Point to first branch	-1.06	-15.07 – 19.49		21.85	-8.82 – 75.89		-15.63	-28.12 – 2.10	
Primary branches/plant	8.27	-12.58 – 24.32		-3.60	-30.53 – 25.45		25.28	3.57 – 42.86	
Secondary branches/plant	4.00	-17.80 – 25.53		-14.64	-39.62 – 13.79		35.69	20.49 – 50.82	
Main shoot length	4.83	-2.49 – 15.84		2.06	-10.58 – 19.15		8.35	0.25 – 16.37	
Point to first siliqua on main shoot	-1.46	-26.27 – 15.69		1.77	-29.85 – 28.21		-3.31	-22.31 – 9.92	
Siliqua length	10.56	-4.84 – 25.47		14.11	-9.93 – 40.00		7.95	-2.56 – 20.51	
Seeds/siliqua	7.73	-3.23 – 20.72		-1.07	-14.77 – 13.91		18.69	8.06 – 29.55	
Total siliquae on the main shoot	3.81	-8.23 – 16.98		-3.29	-21.00 – 17.25		12.92	0.76 – 25.53	
Total number of siliquae/ plant	14.01	-16.92 – 51.34		-4.48	-34.82 – 29.5		42.57	14.54 – 85.71	
Seed yield/plant	41.40	-11.61 – 84.53		23.28	-32.84 – 57.93		67.99	28.46 – 124.28	
Biological yield/plant	28.16	-21.59 – 68.50		14.75	-39.34 – 56.16		46.62	10.83 – 95.20	
Oil content	5.65	1.00 – 8.54		0.77	-7.80 – 7.16		11.16	7.24 – 13.85	
Days to 50% flowering	-5.32	-8.85 – 1.00		-1.10	-7.14 – 4.44		-9.12	-11.54 – -4.49	
1 000-seed weight	2.73	-13.75 – 18.31		16.89	-16.67 – 39.83		-7.52	-15.08 – 17.32	
Harvest index	10.82	-1.10 – 22.92		7.24	-11.59 – 23.31		15.23	4.54 – 22.54	
Seed yield/ha (t)	34.19	11.76 – 58.4		26.07	-2.38 – 66.03		45.15	21.32 – 70.50	

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Table S4. Number of introgression line hybrids (ILHs) having significant mid-parent heterosis (MPH) for seed yield and its contributing traits

Traits	Number of DRMRJ31_ILHs expressing significant mid-parent heterosis (MPH)		Number of PM30_ILHs expressing significant mid-parent heterosis (MPH)	
	positive MPH	negative MPH	positive MPH	negative MPH
Plant height (cm)*	1	2	2	0
Point to first branch (cm)*	4	0	2	2
Primary branches/plant	1	2	4	0
Secondary branches/plant	3	0	2	1
Main shoot length (cm)	3	0	3	0
Point to first siliqua on main shoot (cm)*	3	2	0	1
Siliqua length (cm)	10	1	8	0
Seeds/siliqua	1	2	5	0
Total siliqua on main shoot	0	1	3	0
Total number of siliqua/plant	5	0	4	0
Seed yield per plant (g)	12	0	10	0
Biological yield per plant (g)	10	0	7	1
Oil content (%)	1	3	10	0
Days to 50% flowering*	0	21	0	9
1 000-seed weight (g)	15	0	2	1
Harvest index (%)	2	1	3	0
Seed yield/ha (t)	21	0	11	0

*hybrids expressing significant negative heterosis are desirable; DRMRJ31_ILHs – hybrids generated between ILs of DRMRJ 31 (DRMRJ31_ILs) and DRMRJ 31; PM30_ILHs – hybrids generated between ILs of Pusa Mustard 30 (PM30_ILs) and Pusa Mustard 30

Table S5. Estimation of mode of inheritance of heterotic introgression lines (ILs) of DRMRIJ 31 having introgressed genomic segments of *B. carinata* (BC-4)

DRMRIJ31_ILs	Traits	Mid parent heterosis (%) [§]	DRMRIJ 31	Trait value of ILs	DRMRIJ31_ILs - DRMRIJ 31 <i>t</i> -test	Trait value of DRMRIJ31_ILs	DRMRIJ31_ILs - DRMRIJ 31 <i>t</i> -test	DRMRIJ31_ILs - DRMRIJ31_ILs <i>t</i> -test	Qualitative mode of inheritance
IL-82	SY/ha	30.07*	2.73	2.23	-18.29*	3.23	18.17*	44.63*	+Overdominant, -Recessive
IL-87	SY/ha	45.33*	2.73	1.77	-35.36*	3.27	19.64*	85.09*	+Overdominant, -Recessive
IL-89	SY/ha	50.00*	2.73	2.33	-14.63	3.80	39.03*	62.86*	+Overdominant
IL-90	SY/ha	16.17*	2.73	2.83	3.66	3.23	18.29*	14.12	+Dominant
IL-91	SY/ha	24.62*	2.73	2.47	-9.75	3.24	18.54*	31.35*	+Overdominant
IL-95	SY/ha	43.86*	2.73	1.07	-60.97*	2.73	0	156.24*	-Recessive
IL-97	SY/ha	26.24*	2.73	1.97	-28.05*	2.97	8.54	50.85*	-Recessive
IL-101	SY/ha	31.43*	2.73	1.93	-29.27*	3.07	12.20	58.63*	-Recessive
IL-104	SY/ha	33.85*	2.73	1.60	-41.46*	2.90	6.10	81.25*	-Recessive
IL-105	SY/ha	26.12*	2.73	2.50	-8.54	3.30	20.73*	32.00*	+Overdominant
IL-115	SY/ha	51.73*	2.73	1.13	-58.54*	2.93	7.32	158.83*	-Recessive
IL-119	SY/ha	44.45*	2.73	2.07	-24.39*	3.47	26.83*	67.74*	+Overdominant, -Recessive
IL-120	SY/ha	67.22*	2.73	1.33	-51.22*	3.40	24.39*	155.01*	+Overdominant, -Recessive
IL-121	SY/ha	53.45*	2.73	1.13	-58.54*	2.97	8.54	161.78*	-Recessive
IL-128	SY/ha	35.53*	2.73	2.03	-25.61*	3.23	18.17*	58.86*	+Overdominant, -Recessive
IL-131	SY/ha	38.30*	2.73	1.97	-28.05*	3.25	18.90*	65.25*	+Overdominant, -Recessive
IL-136	SY/ha	29.34*	2.73	2.83	3.66	3.60	31.71*	27.06*	+Overdominant
IL-139	SY/ha	31.68*	2.73	2.23	-18.29*	3.27	19.64*	46.42*	+Overdominant
IL-140	SY/ha	16.47*	2.73	2.93	7.32	3.30	20.73*	12.50	-Recessive
IL-156	SY/ha	73.05*	2.73	1.97	-28.05*	4.07	48.78*	106.78*	+Overdominant, -Recessive
IL-91	HI	-21.23*	25.60	25.27	-1.30	20.03	-21.74*	-20.71*	-Overdominant
IL-95	HI	24.27*	25.60	20.00	-21.88*	28.33	10.68	41.67*	-Recessive

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Table S5 to be continued

DRMRJ31_ILs	Traits	Mid parent heterosis (%) [§]	DRMRJ 31	Trait value of ILs	DRMRJ31_ILs - DRMRJ 31 <i>t</i> -test	Trait value of DRMRJ31_ILs	DRMRJ31_ILs - DRMRJ 31 <i>t</i> -test	DRMRJ31_ILs - DRMRJ31_ILs <i>t</i> -test	Qualitative mode of inheritance
IL-82	TSW	26.09*	4.07	3.60	-11.48	4.83	18.85*	34.26*	+Overdominant
IL-87	TSW	13.04*	4.07	5.90	45.08*	5.63	38.52*	-4.52	+Dominant
IL-89	TSW	15.52*	4.07	5.17	27.05*	5.33	31.15*	3.22	+Dominant
IL-90	TSW	12.54*	4.07	5.23	28.69*	5.23	28.69*	0	+Dominant
IL-92	TSW	22.60*	4.07	4.63	13.93	5.33	31.15*	15.11*	+Overdominant
IL-104	TSW	17.27*	4.07	4.23	4.10	4.87	19.67*	14.96*	+Overdominant
IL-105	TSW	20.75*	4.07	4.77	17.21*	5.33	31.15*	11.89	+Dominant
IL-106	TSW	15.87*	4.07	4.33	6.56	4.87	19.67*	12.31	+Dominant
IL-119	TSW	14.86*	4.07	4.23	4.10	4.77	17.21*	12.60	+Dominant
IL-120	TSW	21.81*	4.07	4.03	-0.82	4.93	21.31*	22.31*	+Overdominant
IL-136	TSW	15.45*	4.07	4.13	1.64	4.73	16.39*	14.52	+Dominant
IL-139	TSW	15.04*	4.07	4.80	18.03*	5.10	25.41*	6.25	+Dominant
IL-140	TSW	17.56*	4.07	4.67	14.75	5.13	26.23*	10.00	+Dominant
IL-156	TSW	24.48*	4.07	3.97	-2.46	5.00	22.95*	26.05*	+Overdominant
IL-158	TSW	23.60*	4.07	3.70	-9.02	4.80	18.03*	29.73*	+Overdominant
IL-87	OC	6.58*	37.13	34.30	-7.63*	38.07	2.51	10.98*	-Recessive
IL-104	OC	-6.01*	37.13	39.47	6.28*	36.00	-3.05	-8.78*	+Recessive
IL-106	OC	-11.97*	37.13	35.80	-3.59	32.10	-13.55*	-10.34*	-Overdominant
IL-110	OC	-5.43*	37.13	33.50	-9.78*	33.40	-10.05*	-0.30	+Dominant
IL-82	BY/pt	50.09*	74.67	68.00	-8.93	107.07	43.39*	57.45*	+Overdominant
IL-87	BY/pt	40.98*	74.67	69.47	-6.96	101.60	36.07*	46.26*	+Overdominant
IL-89	BY/pt	34.93*	74.67	69.80	-6.52	97.47	30.54*	39.64*	+Overdominant
IL-91	BY/pt	39.85*	74.67	76.07	1.87	105.40	41.16*	38.56*	+Overdominant
IL-97	BY/pt	19.26*	74.67	79.73	6.79	92.07	23.30*	15.47	+Dominant
IL-119	BY/pt	39.55*	74.67	73.33	-1.79	103.27	38.30*	40.82*	+Overdominant
IL-120	BY/pt	29.80*	74.67	70.07	-6.16	93.93	25.80*	34.06*	+Overdominant
IL-121	BY/pt	41.38*	74.67	71.13	-4.73	103.07	38.04*	44.89*	+Overdominant
IL-146	BY/pt	29.28*	74.67	82.20	10.09	101.40	35.80*	23.36*	+Overdominant

8 Table S5 to be continued

DRMRIJ31_ILs	Traits	Mid parent heterosis (%) [§]	DRMRIJ 31	Trait value of ILs	DRMRIJ31_ILs - DRMRIJ 31 <i>t</i> -test	Trait value of DMRJ31_ILs	DRMRIJ31_ILs - DRMRIJ 31 <i>t</i> -test	DRMRIJ31_ILs - DRMRIJ31_ILs <i>t</i> -test	Qualitative mode of inheritance
IL-82	SY/pt	57.45*	19.10	13.80	-27.75*	25.90	35.60*	87.68*	+Overdominant
IL-87	SY/pt	45.23*	19.10	13.77	-27.92*	23.87	24.96*	73.37*	+Overdominant
IL-89	SY/pt	50.24*	19.10	15.47	-19.02	25.97	35.95*	67.89*	+Overdominant
IL-95	SY/pt	42.61*	19.10	12.03	-37.00*	22.20	16.23	84.49*	-Recessive
IL-115	SY/pt	24.38*	19.10	10.43	-45.38*	18.37	-3.84	76.04*	-Recessive
IL-119	SY/pt	46.46*	19.10	17.13	-10.30	26.53	38.92*	54.86*	+Overdominant
IL-120	SY/pt	51.91*	19.10	11.40	-40.31*	23.17	21.29	103.22*	-Recessive
IL-121	SY/pt	54.81*	19.10	11.73	-38.57*	23.87	24.96*	103.41*	+Overdominant
IL-128	SY/pt	22.43*	19.10	14.93	-21.82*	20.83	9.07	39.51*	-Recessive
IL-140	SY/pt	21.99*	19.10	23.03	20.59	25.70	34.55*	11.58	+Dominant
IL-146	SY/pt	42.91*	19.10	16.63	-12.91	25.53	33.68*	53.51*	+Overdominant
IL-82	TS/pt	33.98*	291.47	219.27	-24.77*	342.13	17.38	56.04*	-Recessive
IL-119	TS/pt	18.26*	291.47	364.13	24.93*	387.67	33.01*	6.46	+Dominant
IL-120	TS/pt	32.58*	291.47	279.67	-4.05	378.60	29.89*	35.38*	+Overdominant
IL-90	SMS	-11.20*	56.27	44.93	-20.14*	44.93	-20.14*	0	-Dominant
IL-104	SPS	16.37*	15.63	12.27	-21.53*	16.23	3.84	32.34*	-Recessive
IL-115	SPS	-11.47*	15.63	13.13	-15.99*	12.73	-18.55*	-3.05	-Dominant
IL-119	SPS	-9.31*	15.63	14.43	-7.68	13.63	-12.79*	-5.54	-Dominant
IL-82	SL	14.83*	3.87	3.10	-19.83*	4.00	3.45	29.03*	-Recessive
IL-87	SL	11.42*	3.87	3.43	-11.21*	4.07	5.17	18.45*	-Recessive
IL-97	SL	11.76*	3.87	2.93	-24.14*	3.80	-1.72	29.55*	-Recessive
IL-101	SL	14.06*	3.87	4.43	14.65*	4.73	22.41*	6.77	+Dominant
IL-106	SL	15.00*	3.87	4.13	6.89	4.60	18.96*	11.29*	+Overdominant
IL-139	SL	23.38*	3.87	2.83	-26.73*	4.13	6.89	45.88*	-Recessive
IL-141	SL	11.49*	3.87	3.97	2.59	4.37	12.93*	10.08*	+Overdominant
IL-158	SL	16.07*	3.87	3.60	-6.90	4.33	12.07*	20.37*	+Overdominant
IL-97	FSM	-20.00*	8.20	16.80	104.88*	10.00	21.95	-40.48*	+Recessive
IL-105	FSM	-18.90*	8.20	13.67	66.67*	8.87	8.13	-35.12*	+Recessive

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Table S5 to be continued

DRMRIJ31_ILs	Traits	Mid parent heterosis (%) [§]	DRMRIJ 31	Trait value of ILs	DRMRIJ31_ILs - DRMRIJ 31 <i>t</i> -test	Trait value of DRMRIJ31_ILs	DRMRIJ31_ILs - DRMRIJ 31 <i>t</i> -test	DRMRIJ31_ILs - DRMRIJ31_ILs <i>t</i> -test	Qualitative mode of inheritance
IL-106	FSM	20.00*	8.20	8.80	7.32	10.20	24.39*	15.91	+Dominant
IL-128	FSM	25.12*	8.20	5.33	-34.96*	8.47	3.25	58.75*	-Recessive
IL-106	MSL	10.07*	84.13	84.00	-0.16	92.53	9.98*	10.16*	+Overdominant
IL-128	MSL	9.51*	84.13	75.00	-10.86*	87.13	3.57	16.18*	-Recessive
IL-136	MSL	17.75*	84.13	58.20	-30.82*	83.80	-0.40	43.99*	-Recessive
IL-87	SB/pt	32.65*	9.47	9.93	4.93	12.87	35.92*	29.53*	+Overdominant
IL-120	SB/pt	20.60*	9.47	12.87	35.92*	13.47	42.25*	4.66	+Dominant
IL-119	PB/pt	-13.40*	4.03	5.67	40.50*	4.20	4.13	-25.88*	+Recessive
IL-136	PB/pt	-31.83*	4.03	6.33	57.03*	3.53	-12.40	-44.21*	+Recessive
IL-90	PFB	22.59*	54.40	38.27	-29.66*	56.80	4.41	48.43*	-Recessive
IL-91	PFB	20.58*	54.40	56.07	3.06	66.60	22.43*	18.79	+Dominant
IL-91	PH	7.13*	212.27	195.47	-7.91*	218.40	2.89	11.73*	-Recessive
IL-101	PH	-8.08*	212.27	199.67	-5.94	189.33	-10.80*	-5.18	-Dominant
IL-141	PH	-10.35*	212.27	210.87	-0.66	189.67	-10.65*	-10.05*	-Overdominant
IL-82	D50%F	-10.84*	50.33	60.33	19.87*	49.33	-1.99	-18.23*	+Recessive
IL-87	D50%F	-7.40*	50.33	53.33	5.96*	48.00	-4.64	-10.00*	+Recessive
IL-89	D50%F	-5.37*	50.33	49.00	-2.65	47.00	-6.62*	-4.08	-Dominant
IL-90	D50%F	-6.98*	50.33	50.00	-0.66	46.67	-7.28*	-6.67*	-Overdominant
IL-94	D50%F	-7.32*	50.33	45.33	-9.93*	44.33	-11.92*	-2.21	-Dominant
IL-95	D50%F	-6.34*	50.33	44.33	-11.92*	44.33	-11.92*	0	-Dominant
IL-97	D50%F	-6.00*	50.33	49.67	-1.32	47.00	-6.62*	-5.37	-Dominant
IL-101	D50%F	-7.59*	50.33	46.33	-7.95*	44.67	-11.26*	-3.60	-Dominant
IL-104	D50%F	-8.05*	50.33	49.00	-2.65	45.67	-9.27*	-6.80*	-Overdominant
IL-106	D50%F	-5.08*	50.33	48.00	-4.64	46.67	-7.28*	-2.78	-Dominant
IL-115	D50%F	-6.08*	50.33	48.33	-3.97	46.33	-7.95*	-4.14	-Dominant
IL-121	D50%F	-5.15*	50.33	46.67	-7.28*	46.00	-8.61*	-1.43	-Dominant
IL-128	D50%F	-6.71*	50.33	44.00	-12.58*	44.00	-12.58*	0	-Dominant
IL-131	D50%F	-6.90*	50.33	46.33	-7.95*	45.00	-10.60*	-2.88	-Dominant

Table S5 to be continued

DRMRIJ31_ILs	Traits	Mid parent heterosis (%) ^{\$}	DRMRIJ 31	Trait value of ILs	DRMRIJ31_ILs - DMRIJ 31 <i>t</i> -test	Trait value of DMRIJ31_ILs	DRMRIJ31_ILs - DMRIJ 31 <i>t</i> -test	DRMRIJ31_ILs - DMRIJ31_ILs <i>t</i> -test	Qualitative mode of inheritance
IL-136	D50%F	-13.25*	50.33	60.33	19.87*	48.00	-4.64	-20.44*	+Recessive
IL-139	D50%F	-10.76*	50.33	55.00	9.27*	47.00	-6.62*	-14.55*	-Overdominant
IL-140	D50%F	-8.00*	50.33	49.67	-1.32	46.00	-8.61*	-7.38*	-Overdominant
IL-141	D50%F	-10.75*	50.33	52.00	3.31	45.67	-9.27*	-12.18*	-Overdominant
IL-146	D50%F	-7.89*	50.33	51.00	1.32	46.67	-7.28*	-8.50*	-Overdominant
IL-158	D50%F	-5.96*	50.33	44.67	-11.26*	44.67	-11.26*	0	-Dominant

^{\$} – represent the using the Benjamini–Hochberg false discovery rate (FDR) correction

PH – plant height (cm); PFB – point to first branch (cm); PB/pt – primary branches/plant; SB/pt – secondary branches/plant; MSL – main shoot length (cm); FSM – point to first silique on main shoot (cm); SL – silique length (cm); SPS – seeds/silique; SMS – total silique on main shoot; TS/pt – total number of silique/plant; SY/pt – seed yield per plant (g); BY/pt – biological yield per plant (g); OC – oil content (%); D50%F – days to 50% flowering; TSW – 1 000-seed weight (g); HI – harvest index (%); SY/ha – seed yield/ha (t); *significant at $P = 0.05$ of pairwise comparisons using *t*-test; DMRMJ31_IL – *B. carinata*-derived *B. juncea* introgression line in the genetic background of DMRMJ 31; DMRMJ31_ILHs – hybrids generated between ILs of DMRMJ 31 (DRMJ31_ILs) and DMRMJ 31

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Table S6. Estimation of mode of inheritance of heterotic introgression lines (ILs) of Pusa Mustard 30 (PM 30) having introgressed genomic segments of *B. carinata* (BC-4)

PM30_ILs	Traits	Mid parent heterosis (%) §	PM 30	Trait value of ILs	PM30_ILs – PM 30 <i>t</i> -test	Trait value of PM30_ILs	PM30_ILs – PM30_ILs <i>t</i> -test	Qualitative mode of inheritance
IL-168	SY/ha	36.21*	2.03	1.83	-9.84	2.63	29.51*	+Over-dominance
IL-171	SY/ha	28.47*	2.03	2.53	24.59*	2.93	44.26*	+Dominance
IL-179	SY/ha	48.49*	2.03	2.37	16.40	3.27	60.66*	+Over-dominance
IL-180	SY/ha	45.73*	2.03	2.27	11.48	3.13	54.10*	+Over-dominance
IL-181	SY/ha	19.18*	2.03	2.83	39.34*	2.90	42.63*	+Dominance
IL-185	SY/ha	58.40*	2.03	2.13	4.92	3.30	62.30*	+Over-dominance
IL-186	SY/ha	25.55*	2.03	2.53	24.59*	2.87	40.99*	+Dominance
IL-187	SY/ha	34.27*	2.03	2.73	34.43*	3.20	57.38*	+Over-dominance
IL-188	SY/ha	54.38*	2.03	1.77	-13.11	2.93	44.26*	+Over-dominance
IL-190	SY/ha	48.57*	2.03	2.63	29.51*	3.47	70.50*	+Over-dominance
IL-179	HI	19.81*	21.30	20.60	-3.29	25.10	17.84*	+Over-dominance
IL-185	HI	22.92*	21.30	21.17	-0.63	26.10	22.54*	+Over-dominance
IL-170	TSW	18.31*	5.97	5.87	-1.68	7.00	17.32*	+Over-dominance
IL-177	TSW	12.42*	5.97	4.23	-29.05*	5.73	-3.91	-Recessive
IL-168	D50%F	-6.98*	52.00	48.33	-7.05*	46.67	-10.26*	-Dominant
IL-171	D50%F	-5.08*	52.00	46.33	-10.90*	46.67	-10.26*	-Dominant
IL-177	D50%F	-7.74*	52.00	51.33	-1.28	47.67	-8.33*	-Over-dominance
IL-179	D50%F	-6.76*	52.00	46.67	-10.26*	46.00	-11.54*	-Dominant
IL-180	D50%F	-3.75*	52.00	45.67	-12.18*	47.00	-9.62*	-Dominant
IL-181	D50%F	-7.54*	52.00	49.67	-4.49*	47.00	-9.62*	-Over-dominance
IL-182	D50%F	-8.85*	52.00	49.67	-4.49*	46.33	-10.90*	-Over-dominance
IL-187	D50%F	-6.58*	52.00	49.33	-5.13*	47.33	-8.97*	-Over-dominance
IL-190	D50%F	-6.40*	52.00	47.00	-9.62*	46.33	-10.90*	-Dominant
IL-168	OC	7.00*	31.77	35.40	11.44*	35.93	13.12*	+Dominance
IL-170	OC	8.04*	31.77	32.90	3.57	34.93	9.97*	+ Over-dominance
IL-177	OC	8.47*	31.77	34.73	9.34*	36.07	13.54*	+Dominance
IL-179	OC	4.73*	31.77	35.13	10.60*	35.03	10.28*	+Dominance
IL-180	OC	6.22*	31.77	36.33	14.38*	36.17	13.85*	+Dominance
IL-181	OC	4.63*	31.77	35.90	13.01*	35.40	11.44*	+Dominance
IL-182	OC	8.54*	31.77	32.60	2.62	34.93	9.97*	+ Over-dominance

Table S6 to be continued

PM30_ILs	Traits	Mid parent heterosis (%) ^{\$}	PM 30	Trait value of ILs	PM30_ILs – PM 30 <i>t</i> -test	Trait value of PM30_ILs	PM30_ILs – PM 30 <i>t</i> -test	PM30_ILs – PM30_ILs <i>t</i> -test	Qualitative mode of inheritance
IL-185	OC	6.12*	31.77	33.63	5.88*	34.70	9.23*	3.17	+Dominance
IL-187	OC	6.71*	31.77	34.83	9.65*	35.53	11.86*	2.01	+Dominance
IL-190	OC	5.75*	31.77	34.30	7.97*	34.93	9.97*	1.85	+Dominance
IL-168	BY/pt	30.20*	59.73	63.67	6.58	80.33	34.49*	26.18*	+ Over-dominance
IL-171	BY/pt	29.75*	59.73	73.13	22.43	86.20	44.31*	17.87	+Dominance
IL-177	BY/pt	-21.59*	59.73	109.13	82.70*	66.20	10.83	-39.34*	+ Recessive
IL-179	BY/pt	44.08*	59.73	88.33	47.88*	106.67	78.57*	20.75*	+ Over-dominance
IL-180	BY/pt	59.32*	59.73	62.20	4.13	97.13	62.61*	56.16*	+ Over-dominance
IL-186	BY/pt	68.50*	59.73	78.67	31.70*	116.60	95.20*	48.22*	+ Over-dominance
IL-188	BY/pt	34.16*	59.73	79.40	32.92*	93.33	56.25*	17.55	+Dominance
IL-190	BY/pt	56.22*	59.73	70.93	18.75	102.07	70.87*	43.89*	+ Over-dominance
IL-168	SY/pt	50.39*	12.77	12.77	0	19.20	50.39*	50.39*	+ Over-dominance
IL-171	SY/pt	39.98*	12.77	17.00	33.16	20.83	63.18*	22.55	+Dominance
IL-179	SY/pt	74.19*	12.77	17.97	40.73*	26.77	109.66*	48.98*	+ Over-dominance
IL-180	SY/pt	74.53*	12.77	15.77	23.50	24.90	95.04*	57.93*	+ Over-dominance
IL-181	SY/pt	22.65*	12.77	21.97	72.06*	21.30	66.84*	-3.04	+Dominance
IL-182	SY/pt	25.68*	12.77	19.17	50.13*	20.07	57.18*	4.70	+Dominance
IL-185	SY/pt	39.16*	12.77	17.37	36.03*	20.97	64.23*	20.73	+ Over-dominance
IL-186	SY/pt	84.53*	12.77	18.27	43.08*	28.63	124.28*	56.75*	+ Over-dominance
IL-188	SY/pt	56.46*	12.77	15.87	24.28	22.40	75.46*	41.18*	+ Over-dominance
IL-190	SY/pt	62.19*	12.77	15.27	19.58	22.73	78.07*	48.91*	+ Over-dominance
IL-179	TS/pt	46.36*	233.80	319.80	36.78*	405.13	73.28*	26.68*	+ Over-dominance
IL-180	TS/pt	30.70*	233.80	322.80	38.07*	363.73	55.57*	12.68	+Dominance
IL-186	TS/pt	44.46*	233.80	294.87	26.12	381.87	63.33*	29.50*	+ Over-dominance
IL-188	TS/pt	51.34*	233.80	340.00	45.42*	434.20	85.71*	27.71*	+ Over-dominance
IL-180	SMS	11.78*	43.87	54.07	23.25*	54.73	24.77*	1.23	+Dominance
IL-185	SMS	15.08*	43.87	47.67	8.66	52.67	20.06*	10.49	+Dominance
IL-186	SMS	16.98*	43.87	43.67	-0.46	51.20	16.72*	17.25*	+ Over-dominance
IL-168	SPS	10.55*	11.17	12.53	12.24*	13.10	17.31*	4.52	+Dominance
IL-170	SPS	9.70*	11.17	12.53	12.24*	13.00	16.42*	3.72	+Dominance

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Table S6 to be continued

PM30_ILs	Traits	Mid parent heterosis (%) §	PM 30	Trait value of ILs	PM30_ILs – PM 30 <i>t</i> -test	Trait value of PM30_ILs	PM30_ILs – PM 30 <i>t</i> -test	PM30_ILs – PM30_ILs <i>t</i> -test	Qualitative mode of inheritance
IL-180	SPS	20.72*	11.17	12.80	14.63*	14.47	29.55*	13.02*	+ Over-dominance
IL-187	SPS	11.47*	11.17	13.83	23.88*	13.93	24.78*	0.72	+ Dominance
IL-190	SPS	15.59*	11.17	11.50	2.98	13.10	17.31*	13.91*	+ Over-dominance
IL-177	SL	12.68*	3.90	3.20	-17.95*	4.00	2.56	25.00*	-Recessive
IL-179	SL	12.76*	3.90	4.20	7.69	4.57	17.09*	8.73	+ Dominance
IL-181	SL	10.32*	3.90	4.50	15.38*	4.63	18.80*	2.96	+ Dominance
IL-182	SL	17.50*	3.90	4.10	5.13	4.70	20.51*	14.63*	+ Over-dominance
IL-190	SL	25.47*	3.90	3.17	-18.80*	4.43	13.67*	40.00*	+ Over-dominance
IL-179	FSM	-26.27*	8.07	8.93	10.74	6.27	-22.31*	-29.85*	-Over-dominance
IL-170	MSL	8.63*	81.47	70.00	-14.08*	82.27	0.98	17.52*	-Recessive
IL-182	MSL	15.84*	81.47	78.07	-4.17	92.40	13.42*	18.36*	+ Over-dominance
IL-186	MSL	15.48*	81.47	76.60	-5.97	91.27	12.03*	19.15*	+ Over-dominance
IL-177	SB/pt	-17.80*	8.13	17.33	113.12*	10.47	28.69	-39.62*	+Recessive
IL-180	SB/pt	25.53*	8.13	10.67	31.15*	11.80	45.08*	10.62	+ Dominance
IL-190	SB/pt	23.60*	8.13	9.67	18.85	11.00	35.25*	13.79	+ Dominance
IL-171	PB/pt	18.26*	3.73	3.93	5.36	4.53	21.43*	15.25	+ Dominance
IL-179	PB/pt	24.19*	3.73	4.53	21.43*	5.13	37.50*	13.24	+ Dominance
IL-180	PB/pt	24.32*	3.73	3.67	-1.78	4.60	23.22*	25.45*	+ Over-dominance
IL-186	PB/pt	19.40*	3.73	5.20	39.29*	5.33	42.86*	2.56	+ Dominance
IL-170	PFB	19.49*	76.33	39.27	-48.56*	69.07	-9.52	75.89*	-Recessive
IL-179	PFB	-14.40*	76.33	51.87	-32.05*	54.87	-28.12*	5.78	- Dominance
IL-185	PFB	14.27*	76.33	60.07	-21.31*	77.93	2.10	29.74*	- Recessive
IL-186	PFB	-15.07*	76.33	66.53	-12.84*	60.67	-20.52*	-8.82	- Dominance

§ – represent the using the Benjamini–Hochberg false discovery rate (FDR) correction

PH – plant height (cm); PFB – point to first branch (cm); PB/pt – primary branches/plant; SB/pt – secondary branches/plant; MSL – main shoot length (cm); FSM – point to first siliqua on main shoot (cm); SL – siliqua length (cm); SPS – seeds/siliqua; SMS – total siliqua on main shoot; TS/pt – total number of siliqua/plant; SY/pt – seed yield per plant (g); BY/pt – biological yield per plant (g); OC – oil content (%); D50%F – days to 50% flowering; TSW – 1 000-seed weight (g); HI – harvest index (%); SY/ha – seed yield/ha (t);

*significant at $P = 0.05$ of pairwise comparisons using *t*-test; PM30_IL – *B. carinata*-derived *B. juncea* introgression line in the genetic background of Pusa Mustard 30; PM30_ILs

– hybrids generated between ILs of Pusa Mustard 30 (PM30_ILs) and Pusa Mustard 30

Table S7. Identified candidate genes in heterotic genomic segments introgressed from *B. carinata* (BC-4) into *B. juncea* cultivars DRMRIJ 31

DRMRIJ31_ILs	SNP ID	<i>A. thaliana</i> ortholog	Gene name	Function	Already known to influence trait(s)	References
IL136	B7_55230799	AT1G19850	<i>ARF5</i>	Auxin Response Factor 5	Thousand seed weight (g)	Liu et al. (2015); Mathur et al. (2022)
IL 82, IL 87, IL136	B2_52492605	AT4G11260	<i>SGT1B, ETA3</i>	Enhancer Of TIR1-1 Auxin Resistance 3		
IL 82, IL 115, IL 121, IL 128	A1_11494423	AT3G16857	<i>ARR1</i>	Response regulator 1	Thousand seed weight (g)	Dhaka et al. (2017)
IL 87, IL 115, IL 119, IL 156	B7_31937306	AT1G72010	<i>TCP22</i>	TCP Domain Protein 22		
IL 95, IL 104, IL105, IL 115, IL156	B5_50104581	AT3G15030	<i>MEE35, TCP4</i>	TCP Family Transcription Factor 4, Maternal Effect Embryo Arrest 35	Thousand seed weight (g)	Aakanksha et al. (2021); Mathur et al. (2022)
IL136	A9_35329789	AT5G41240	<i>GSTT2</i>	Glutathione S-Transferase THETA 2	Seed yield, thousand seed weight(g)	Kumar et al. (2022)
IL 82, IL 87, IL136	B2_36130560	AT2G13790	<i>BKK1</i>	BR11- Associated Kinase 7	Seed size	Jiang et al. (2013)
IL136	A9_8682715	AT1G62760	<i>AtPMEI10</i>	Pectin Methyltransferase 10		
IL 82, IL136	B3_13463145	AT2G39700	<i>ATEXPA4</i>	Expansin A4	Thousand seed weight (g)	Mathur et al. (2022)
IL 97, IL136	B7_53422098	AT4G26640	<i>AtWRKY20</i>	WRKY family transcription factors		
IL 82	A5_28360955	AT3G14067	<i>SASP/SBT1.4</i>	Senescence-associated subtilisin protease	Secondary branches, Total siliqua per plant	Martinez et al. (2015)
IL 95	B5_33601027	AT2G05920	<i>SBT1.8</i>	SUBTILASE 1.8	Secondary branches, Total siliqua per plant	Martinez et al. (2015)
IL120	B4_57181897	AT1G75820	<i>ATCLV1, FLO5</i>	Clavata 1	Total siliqua per plant	Cai et al. (2016)
IL 82, IL 87, IL 128, IL 136, IL 140	B2_65115276	AT1G76430	<i>PHT1;9</i>	phosphate transporter 1;9	Point to first branch, seed weight, primary branches	Shi et al. (2013)
IL 97, IL 136	B7_56513236	AT1G12370.2	<i>PHR1, UVR2</i>	UV RESISTANCE 2, photolyase 1	Point to first branches	Shi et al. (2013)
IL 82, IL 115	B2_5857235	AT4G26120	<i>BTB/POZ</i>	BTB/POZ domain-containing protein NPR2)	Days to flowering	Patel et al. (2025)
IL 82, IL95, IL 97, IL136	B7_43692939	AT1G25440	<i>BBX15</i>	B-Box Domain Protein 15	Days to flowering	Aakanksha et al. (2021)
IL 128, IL 140	B3_5025604	AT5G18960	<i>FRS12</i>	FAR1-related sequence 12	Days to flowering	Patel et al. (2025)

DRMRIJ31_IL – *B. carinata*-derived *B. juncea* introgression line in the genetic background of DRMRIJ 31; SNP ID B7_55230799 – first letter in SNP ID denotes genome B, after this number denotes chromosome number; and the last portion denotes the position of SNP in base pairs

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Table S8. Identified candidate genes in heterotic genomic segments introgressed from *B. carinata* (BC-4) into *B. juncea* cultivars Pusa Mustard 30

PM30_ILs	SNP ID	<i>A. thaliana</i> ortholog	Gene name	Function	Already known to influence trait(s)	References
IL168, IL 170, IL180, IL 181, IL190	A4_14198854	AT2G24790	<i>BBX4, ATCOL3</i>	B-Box Domain Protein 4, CONSTANS-Like 3	Days to 50% flowering	Aakanksha et al. (2021)
IL 170, IL 177, IL180, IL 181, IL 185	B4_9922543	AT1G80010	<i>FRS8</i>	FAR1-related sequence 8	Days to flowering	Patel et al. (2025)
IL168	A1_7869145	AT2G40540	<i>ATKT2</i>	Potassium Transporter 2		
IL168, IL180	A9_8798604	AT1G62640	<i>KAS III</i>	3-Ketoacyl-Acyl Carrier Protein Synthase III		
IL168, IL185, IL 177, IL 181	B7_49573116	AT4G34131	<i>UGT73B3</i>	UDP-Glucosyl Transferase 73B3	Oil content (%)	Kumar et al. (2022)
IL180, IL185, IL 181	A3_22305061	AT3G50740	<i>UGT72E1</i>	UDP-Glucosyl Transferase 72E1		
IL185, IL187, IL190	B3_4746117	AT4G27140	<i>SESA1</i>	Seed Storage Albumin 1		
IL168, IL180, IL 170, IL 177, IL 181	A9_11281228	AT1G55050	<i>SANT4</i>	SANT domain	Thousand seed weight (g)	
IL187	B4_13959821	AT1G06070	<i>bZIP69</i>	Basic Leucine-Zipper 69	Thousand seed weight (g)	Mathur et al. (2022)
IL 177, IL 180, IL 181	B3_14780775	AT1G34760	<i>GRF 11</i>	General regulatory factor 11	Thousand seed weight (g)	Dhaka et al. (2017)
IL185, IL 170	B6_16324111	AT1G09770	<i>ATMYBCDC5</i>	<i>Arabidopsis thaliana</i> Cell Division Cycle 5	Thousand seed weight (g)	Aakanksha et al. (2021); Mathur et al. (2022)
IL168, IL180, IL187, IL190, IL 181	A4_13933042	AT2G24450	<i>FLA3</i>	FASCICLIN-Like Arabinogalactan Protein 3 Precursor	Siliqua length (cm)	Wang et al. (2019)

PM30_ILs – *B. carinata*-derived *B. juncea* introgression lines in the genetic background of cultivar Pusa Mustard 30; SNP ID A4_14198854 – first letter in SNP ID denotes Genome A, after this number denotes chromosome number, and last portion denotes position of SNP in base pairs

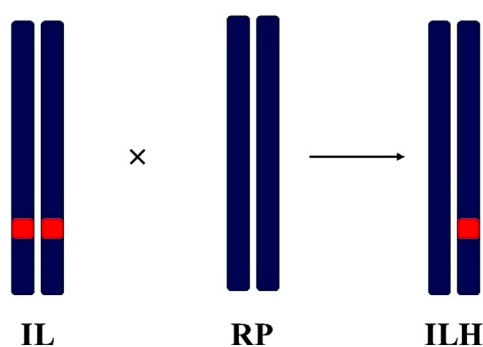


Figure S1. Development of Introgression lines hybrid (ILH) by hybridising IL and the recipient parent (RP)

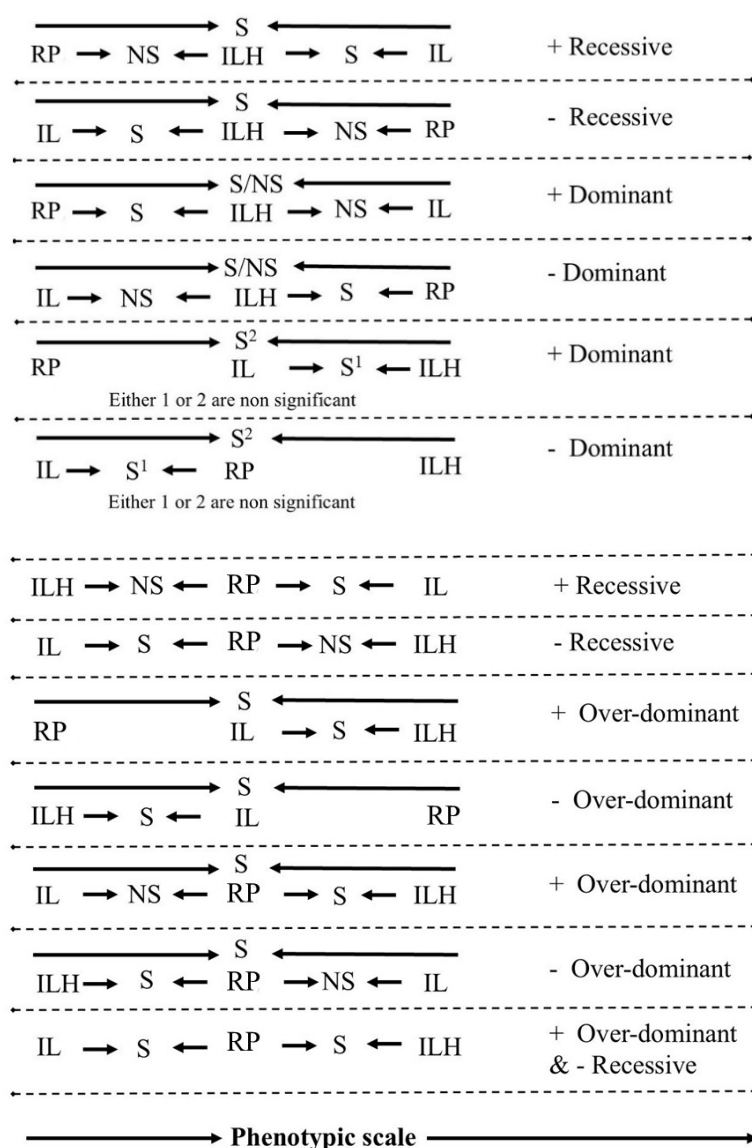


Figure S2. Schematic illustration to unravel the genetic basis of heterosis*

IL – introgression line; ILH – introgression line hybrid; RP – *B. juncea* recipient parent; S – significant @5%, NS – non-significant

*Heterotic effects of introgressed segments were classified as dominant, recessive, and over-dominant based on pairwise comparisons of phenotypic means among ILHs, corresponding ILs, and the recipient parent

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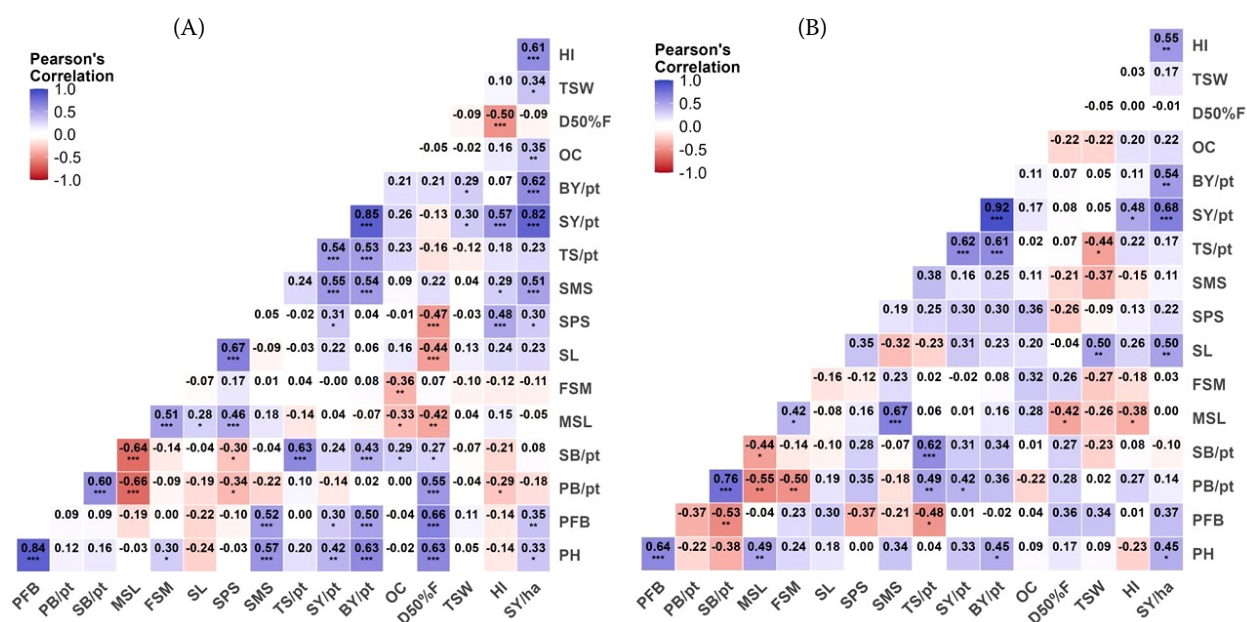


Figure S3. Correlation coefficients between different agro-morphological traits in the genetic background of (A) DR-MRIJ 31 and (B) Pusa Mustard 30

*, **, ***Significant at $P = 0.05, 0.01, 0.001$; PH – plant height (cm); PFB – point to first branch (cm); PB – primary branches/plant; SB – secondary branches/plant; MSL – main shoot length (cm); FSM – point to first silique on main shoot (cm); SL – silique length (cm); SPS – seeds/silique; SMS – total silique on main shoot; TS/pt – total number of silique/plant; SY/pt – seed yield per plant (g); BY/pt – biological yield per plant (g); OC – oil content (%); D50%F – days to 50% flowering; TSW – 1 000-seed weight (g); HI – harvest index (%); SY/ha – seed yield/ha (t)

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