

Electronic Journal of Plant Breeding

Research Article

Assessment of genetic relationship among diverse Indian mustard (*Brassica juncea* L.) genotypes using XLSTAT

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Abstract

This research was conducted to study the genetic relationship between eleven quantitative traits of 95 Indian mustard (*Brassica juncea* L.) genotypes. The experiment material was evaluated in paired rows of 4 m length at Research Area of Oilseeds Section, Department of Genetics and plant breeding, CCS HAU, Hisar during Rabi, 2017-18. All the recommended package of practices was followed to raise the healthy crop. Maximum variation was observed for seed yield per plant followed by the number of secondary branches per plant, the number of primary branches per plant, 1000-seed weight and the number of siliqua on main shoot. Correlation studies revealed that seed yield per plant was positively and significantly associated with plant height, the number of primary and secondary branches per plant, and the number of siliqua on main shoot length. Selection based on these traits would ultimately improve seed yield. Four ideal genotypes viz., DRMRIJ-14-261, DRMRIJ-15-52, DRMRIJ-15-148 and M 5 were identified for the traits such as medium maturity, the number of primary branches per plant, the number of seeds per siliqua and 1000-seed weight in this study. These genotypes can be used as source lines in breeding programme for obtaining desirable segregates.

Keywords

Descriptive statistics, genetic relationship and Indian mustard (*Brassica juncea* L.)

INTRODUCTION

Brassica juncea (L.) commonly known as 'Indian mustard', is a natural amphidiploid ($2n = 36$) and one of the most important oilseed crops of the country. It occupies considerably large area among the *Brassica* group of oilseeds crop. It is grown both in tropical and subtropical countries. In India, it is predominantly cultivated in Rajasthan, U.P., M.P., Haryana, Gujarat, West Bengal, Assam and Bihar states. The yield of rapeseed-mustard was 1176 kg/ha as compared to 955 kg/ha of the total oilseeds (Singh, 2014). It contributes more than 80% to the total rapeseed-mustard production in the country and is an important component in the oilseed sector (Vinu *et al.*, 2013).

In India *per capita* consumption of edible oil is likely to reach 23- 43 kg by 2030 from the present level of 13.4 kg (Singh, 2014; RamAvtar *et al.*, 2016). This gap is increasing day by day as the breeding methodologies for Indian

mustard improvement remained confined to selection and recombination followed by selection (Mekonnen *et al.*, 2014). To meet out the present yield requirements, there is an urgent need to increase the yield potential of *B. juncea*. The yield is complex in nature and depends on many other morphological characteristics, most of which are inherited quantitatively and highly affected by the environment, so direct selection for yield alone restricts the selection efficiency and eventually results in minimal success in improving it. So, it is important to determine the contribution of each trait in order to give more importance to those with the greatest effect on the seed yield (Tunc Turk and Ciftci, 2007). The Yield component characters demonstrate the relation between themselves and also with the yield. Many research groups have indicated a positive and highly significant correlation between siliqua per plant and yield per plant; seed yield with plant height, branches and siliqua per plant (Tunc Turk

and Ciftci, 2007; Sandhu and Gupta, 1996). Thus, the study of correlation between yield and its components is of primary importance in formulating the selection criteria under the crop improvement (Sarawgi *et al.*, 1997). The selection of any desirable trait is usually preformed on the basis of the plant's phenotypic importance that in turn determined partially by the genotype. This is inherited and partially non heritable by the environment. It is therefore necessary to know the different components of the yield and its mutual correlation with other independent traits. This is because, if it is based on some components that are less sensitive to the environment, selection would be more efficient. The present study was therefore conducted to assess the association analysis for yield contributing traits in Indian mustard (*Brassica juncea* L.).

MATERIALS AND METHODS

To undertake the study on association between seed yield and its contributing traits in Indian mustard, all 95 genotypes of Indian mustard were grown in paired rows of 4 m length with a spacing of 30 x 10 cm (row x plant) at Research Area of Oilseeds Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar during Rabi, 2017-18. **Appendix-I** include an overview of the genotypes. All the recommended package of practices was followed to raise the healthy crop. Observations were recorded on five random and competitive plants for eleven

quantitative traits viz; days to maturity, plant height (cm), the number of primary branches per plant, the number of secondary branches per plant, the number of siliqua on main shoot, main shoot length (cm), siliqua length (cm), the number of seeds per siliqua, seed yield per plant (g), 1000-seed weight (g) and oil content (%). Data on days to maturity was recorded on plot basis. Number of seeds per siliqua was estimated on 10-15 siliquae plucked from main shoot of each of five plants. One thousand seeds were counted from random bulk of each genotype and weighed. The oil content of seeds was determined by the method of AOAC (1995). Statistical analyses have been done with the help of XLSTAT software v5.03 (2014).

RESULTS AND DISCUSSION

Descriptivestatisticalanalysis offered detailed explanations of the data set under study in a manageable form, which showed the basic characteristics of all the traits studied. Mean, range and coefficient of variation for all the 11 traits are presented in **Table 1**. Wide range of variation was observed for most of the traits like seed yield per plant (CV=36.68 %), secondary branches per plant (CV=27.21 %), primary branches per plant (CV=22.62 %), 1000-seed weight (CV=22.34 %) and the number of siliqua on main shoot (CV=21.12 %). Thus, it indicates ample scope for further improvement through simple selection for different quantitative traits for mustard improvement. The variability

Table 1. Mean, range and coefficient of variation (CV %) for 11 different traits in Indian mustard

Characters	Minimum	Maximum	Mean	CV (%)
Days to maturity	134.00	141.00	138.07	1.00
Plant height (cm)	154.30	264.30	208.91	10.32
Number of primary branches	3.30	9.60	5.56	22.62
Number of secondary branches	6.00	23.00	12.91	27.21
Number of siliqua on main shoot	25.60	84.60	50.77	21.12
Main shoot length (cm)	48.60	105.60	79.74	15.56
Siliqua length (cm)	2.40	5.00	3.61	14.30
Seeds per siliqua	8.20	18.10	12.99	13.52
Seed yield (g)	5.00	38.67	16.42	36.68
1000-seed weight (g)	1.82	5.84	3.89	22.34
Oil content (%)	37.80	40.70	38.84	1.71

for days to maturity was very low, it ranged from 134 to 141 days with an average of 138.07 days. Pusa Agrani, RC-38 and NPJ-112 were the early maturing genotypes (≤ 135 days). Plant height ranged from 154.3 cm to 264.3 cm with an average value of 208.91 cm. Low plant height is desirable in mustard due to ease in agronomic practices hence genotypes Pusa Agrani, NPJ-112, MST II 14-2 and EC 27-4 may be used as donor for this trait. Number of primary branches per plant varied from 3.30 to 9.60 with an average of 5.56 while, the number of secondary branches per plant ranged from 6.0 to 23.0 with an average value of 12.91. The genotypes viz; Pusa Kishan, DRMRIJ-14-272 and M-53-B can be used as source lines for both traits i.e. the primary branches per plant as well as the secondary branches per plant. Main shoot length is considered as the most important fruiting zone in *B.*

juncea. Hence, its length and the number of siliqua on main shoot are desirable traits for increasing seed yield. In the present study, main shoot length varied from 48.60 to 105.60 cm, while number of siliqua on main shoot ranged from 25.60 to 84.60. Six genotypes viz. M 16, DRMRIJ-15-104, RC-14, M-53-B, RC-106 and YS-7 had more than 100 cm long main shoot; however, the genotypes bearing maximum number of siliqua (>70) on main shoot were DRMRIJ-14-139, RC-15, DRMRIJ-15-104, M 16 and EC-29-2. Number of seeds per siliqua ranged from 8.20 to 18.10. The variability for seed yield per plant was very high; it varied from 5.00 g to 38.67 g with an average of 16.42 g. Seven genotypes viz., RC-273, DRMRIJ-15-85, RC-53, M 16, YS-7, M 75 and RC-275 had more 26.00 g seed yield per plant, on other hand 12 genotypes had 1000-seed weight more than 5.00 g. Such results are

in concurrence with the results of Singh *et al.*, 2013. None of the genotypes was found to be most promising collectively for all the quantitative traits. However, some genotypes could be identified as promising for different traits (**Table 2**). Based upon multiple trait superiority, four ideal genotypes identified in this study were DRMRIJ-14-261 (136 days maturity, 17 seeds/ siliqua, 5.42 g 1000-seed weight), DRMRIJ-15-52 (166 cm plant height, 16

seeds/siliqua, 40.40 % oil content), DRMRIJ-15-148 (5 cm siliqua length, 15 seeds/ siliqua, 5.50 g 1000-seed weight) and M 5 (8 primary branches, 25 g seed yield, 40 % oil content).

Pearson correlation coefficients showing pair-wise associations between the studied characters of Indian mustard genotypes as presented in **Table 3**.

Table 2. List of promising genotypes of Indian mustard for different traits

Characters	No. of genotypes	Name of genotypes
Days to maturity (≤ 136 days)	12	Pusa Agrani, RC-38, NPJ-112, RC-51, DRMRIJ-14-30, DRMRIJ-14-261, RC-106, RC-18, Pusa Barani, DRMRIJ-15-251, Pusa Tarak and M 47 B line
Plant height (< 176 cm)	10	Pusa Agrani, NPJ-112, MST II 14-2, EC 27-4, DRMRIJ-15-52, EC-27-2, Pusa Tarak, M 62, EJ-17 and Pusa Vijay
Number of primary branches (> 8)	6	M 20, Pusa Kishan, DRMRIJ-14-272, M-53-B line, M 5 and M 75
Number of secondary branches (> 19)	7	M-53-B, DRMRIJ-15-109, M 74, DRMRIJ-14-272, DRMRIJ-15-95, Pusa Kishan and RC-273
Number of siliqua on main shoot (> 70)	5	DRMRIJ-14-139, RC-15, DRMRIJ-15-104, M 16 and EC-29-2
Main shoot length (> 100 cm)	6	M 16, DRMRIJ-15-104, RC-14, M-53-B line, RC-106 and YS-7
Silique length (≥ 5.0 cm)	3	SEJ-8, NPJ-161 and DRMRIJ-15-148
Seeds per siliqua (> 15)	12	LES-39, DRMRIJ-14-261, EC 61-36-1, EC 27-4, RC-18, DRMRIJ-15-03, DRMRIJ-15-52, EC 62-1, DRMRIJ-15-123, RC-14, DRMRIJ-15-148 and M 84
Seed yield (> 24.0 g)	9	RC-273, DRMRIJ-15-85, RC-53, M 16, YS-7, M 75, RC-275, M 5 and Pusa Vijay
1000-seed weight (> 5.0 g)	12	DRMRIJ-14-278, Pusa Vijay, DRMRIJ-15-148, EJ-17, DRMRIJ-14-261, DRMRIJ-15-85, RC-110, DRMRIJ-15-95, DRMRIJ-14-30, M 84, DRMRIJ-14-139 and DRMRIJ-14-137
Oil content (> 40.0 %)	9	RC-51, DRMRIJ-15-123, M 34, RC-275, M 61, EC 62-1, DRMRIJ-15-52, DRMRIJ-14-66 and M 5

Table 3. Estimation of Pearson correlation coefficient (above diagonal) and p values (below diagonal) among different quantitative traits of Indian mustard

Characters	DM	PH	PBr	SBr	SqMS	MSL	SqL	S/Sq	SY	TW	OC
DM		0.460**	0.062	-0.020	0.257*	-0.082	-0.179	-0.186	-0.030	-0.191	0.086
PH	< 0.001		0.321**	0.131	0.505**	0.050	-0.235*	-0.237*	0.269**	-0.205*	0.130
PBr	0.548	0.002		0.631**	0.077	-0.230*	-0.177	-0.223*	0.371**	-0.046	-0.173
SBr	0.847	0.205	< 0.001		0.051	-0.103	-0.217*	-0.050	0.533**	-0.120	-0.172
SqMS	0.012	< 0.001	0.458	0.623		0.575**	-0.260*	-0.171	0.206*	-0.101	0.064
MSL	0.430	0.629	0.025	0.319	< 0.001		0.12	0.192	0.105	0.219*	0.007
SqL	0.083	0.022	0.086	0.035	0.011	0.245		0.335**	0.023	0.415**	-0.004
S/Sq	0.072	0.021	0.029	0.633	0.097	0.062	0.001		0.006	-0.174	0.025
SY	0.775	0.008	0.000	< 0.001	0.046	0.313	0.826	0.956		0.175	0.025
TW	0.064	0.046	0.656	0.246	0.331	0.033	< 0.001	0.091	0.089		-0.077
OC	0.407	0.209	0.094	0.095	0.539	0.948	0.971	0.814	0.810	0.457	

Note: DM = Days to maturity, PH = Plant height (cm), PBr = Number of Primary branches per plant, SBr = Number of secondary branches per plant, SqMS = Number of siliqua on main shoot, MSL = Main shoot length (cm), SqL = Silique length (cm), S/Sq = Seeds per siliqua, SY = Seed yield (g), TW = 1000-seed weight (g) and OC = Oil content (%)

Seed yield per plant was highly significant and positively associated with plant height ($r=0.296$, $p=0.008$), primary branches per plant ($r=0.371$, $p=0.000$), secondary branches per plant ($r=0.533$, $p<0.001$) and the number of siliqua on main shoot ($r=0.206$, $p=0.046$). The association between these characters with seed yield per plant is presented in **Fig. 1**. Generally, the positive associations among these traits suggest the prospect of improving these important yield-attributing characters concurrently. The present study is supported by the previous work done by Akbar

et al., 2003; Hasan *et al.*, 2014; Shekhawat *et al.*, 2014; Banerjee *et al.*, 2017 and Rout *et al.*, 2018 who indicated a positive relationship of different intensity between seed yield per plant and other seed yield related traits. Singh *et al.* (2003) and Lodhi *et al.* (2014) also observed such positive association of seed yield with siliqua on main shoot and plant height. The traits like main shoot length, siliqua length, the number of seeds per siliqua, 1000-seed weight and oil content had a non-significant and positive association with seed yield per plant.

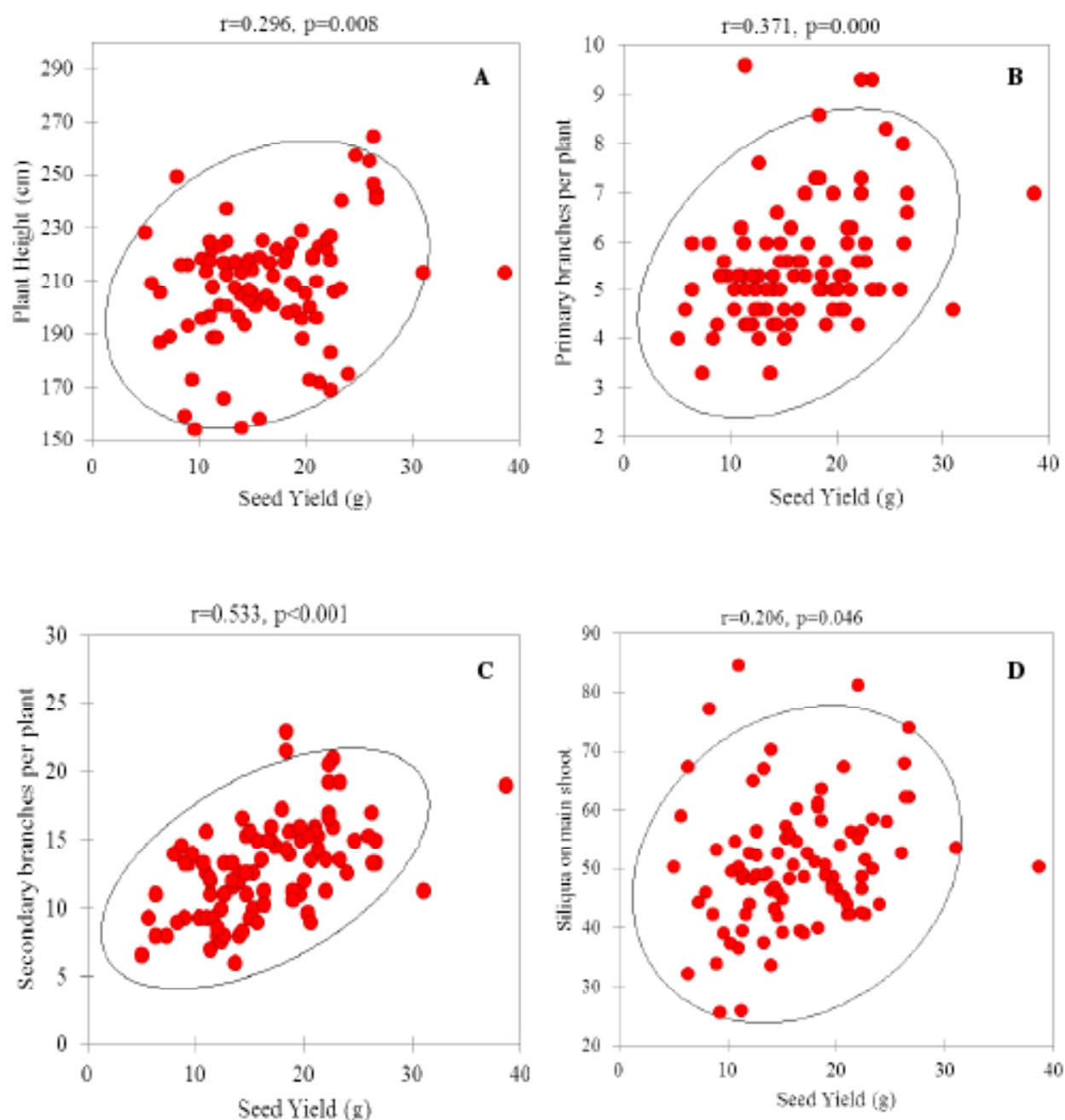


Fig. 1. Graphical representation of correlation between seed yield with plant height (A), primary branches per plant (B), secondary branches per plant (C) and number of siliqua on main shoot (D).

Appendix-I. List of 95 germplasm accessions of Indian mustard used in present study

Sr. No.	Genotypes	Sr. No.	Genotypes	Sr. No.	Genotypes
1	DRMRIJ-14-23	33	LES-43	65	Pusa Jagannath
2	DRMRIJ-14-30	34	LET-17	66	Pusa Kishan
3	DRMRIJ-14-65	35	M 5	67	Pusa Tarak
4	DRMRIJ-14-66	36	M 13	68	Pusa Vijay
5	DRMRIJ-14-99	37	M 16	69	RC-8
6	DRMRIJ-14-137	38	M 20	70	RC-12
7	DRMRIJ-14-139	39	M 22	71	RC-14
8	DRMRIJ-14-261	40	M 27	72	RC-15
9	DRMRIJ-14-272	41	M 28	73	RC-18
10	DRMRIJ-14-278	42	M 34	74	RC-20
11	DRMRIJ-15-03	43	M 37	75	RC-25
12	DRMRIJ-15-52	44	M 47 B line	76	RC-38
13	DRMRIJ-15-85	45	M 49	77	RC-46
14	DRMRIJ-15-95	46	M 61	78	RC-47
15	DRMRIJ-15-104	47	M 62	79	RC-51
16	DRMRIJ-15-108	48	M 65	80	RC-53
17	DRMRIJ-15-109	49	M 67	81	RC-81
18	DRMRIJ-15-123	50	M 74	82	RC-106
19	DRMRIJ-15-133	51	M 75	83	RC-107
20	DRMRIJ-15-143	52	M 78	84	RC-110
21	DRMRIJ-15-148	53	M 80	85	RC-111
22	DRMRIJ-15-150	54	M 81	86	RC-112
23	DRMRIJ-15-251	55	M 82	87	RC-116
24	EC 28	56	M 84	88	RC-142
25	EC 27-2	57	M 23-B line	89	RC-162
26	EC 27-4	58	M 53-B line	90	RC-175
27	EC 29-2	59	MST II 14-2	91	RC-273
28	EC 61-36-1	60	NPJ-112	92	RC-275
29	EC 62-1	61	NPJ-139	93	SEJ-8
30	EJ-17	62	NPJ-161	94	TN-3
31	LES-1-27	63	Pusa Agrani	95	YS-7
32	LES-39	64	Pusa Barani		

Days to maturity exhibited highly significant and positive correlation with plant height ($r=0.460$, $p<0.001$) and the number of siliqua on main shoot length ($r=0.257$, $p=0.012$), while it showed non-significant correlation with other characters. Such results are in concurrence with the results of Roy *et al.*, 2015; Vermai *et al.*, 2016; Banerjee *et al.*, 2017 and Rout *et al.*, 2018. They found positive association of days to maturity with morpho-physiological and seed yield related traits. Plant height showed a highly significant and positive correlation with primary branches per plant ($r=0.321$, $p=0.002$), the number of siliqua on main shoot length ($r=0.505$, $p<0.001$) and seed yield per plant ($r=0.269$, $p=0.008$) whereas, it was negatively associated with siliqua length ($r=-0.235$, $p=0.022$), the number of seeds per siliqua ($r=-0.237$, $p=0.021$) and 1000-seed weight ($r=-0.205$, $p=0.046$). Number of primary branches per plant had a positive and significant association with the number of secondary branches per plant ($r=0.631$, $p<0.001$) and seed yield per plant ($r=0.371$, $p=0.000$).

On the other hand it was negatively associated with main shoot length ($r=-0.230$, $p=0.025$) and the number of seeds per siliqua ($r=-0.223$, $p=0.029$). Such results are in concurrence with those obtained by Lodhi *et al.*, 2014; Banerjee *et al.*, 2017 and Rout *et al.*, 2018.

Number of secondary branches per plant exhibited significant and negative association with siliqua length ($r=-0.260$, $p=0.035$) and positive association with seed yield per plant ($r=0.533$, $p<0.001$). Supporting to our study, Ramanjaneyulu and Giri (2007) reported significant positive association between secondary branches per plant and seed yield in Indian mustard, which was supported later by Lodhi *et al.* (2014) and Banerjee *et al.* (2017). Number of siliqua on main shoot had a positive and significant correlation with main shoot length ($r=0.575$, $p<0.001$), seed yield per plant ($r=0.206$, $p=0.046$) and the negative association with siliqua length ($r=-0.260$, $p=0.011$). Similar results were also reported by Rout *et al.*, 2018. Generally,

the positive association between the number of siliqua per plant and seed yield was reported in plenty of publications in Indian mustard (Akbar *et al.*, 2003; Hasan *et al.*, 2014; Shekhawat *et al.*, 2014). Significant positive correlation was observed between main shoot length and 1000-seed weight ($r=0.219$, $p=0.033$). Siliqua length showed a significant and positive correlation with the number of seeds per siliqua ($r=0.335$, $p=0.001$) and 1000-seed weight also ($r=0.415$, $p<0.001$). Thus, it can be inferred that by improving these traits through selection either alone or in combination, will result in improvement of seed yield in mustard. Similar results were also reported by Roy *et al.*, 2015; Vermai *et al.*, 2016; Rout *et al.*, 2018.

From the above discussion, it may be concluded that differential association was observed among the component characters. The presence of significant and positive association of seed yield with characters plant height, primary branches per plant, secondary branches per plant and the number of siliqua on main shoot length, revealed that the selection based on these traits would ultimately improve seed yield. It is also suggested that hybridization of genotypes possessing combination of above characters is most useful for obtaining desirable high yielding segregates. Four ideal genotypes were identified in present study which was DRMRIJ-14-261, DRMRIJ-15-52, DRMRIJ-15-148 and M 5.

REFERENCES

- Akbar, M., Mahmood, T., Yaqub, M., Anwar, M., Ali, M. and Iqbal, N. 2003. Variability, correlation and path coefficient studies in summer mustard (*Brassica juncea* L.). *Asian J. Plant Sci.*, **2**(9): 696–698. [\[Cross Ref\]](#)
- AOAC 1995. *Official methods of analysis. Association of official agricultural chemist, 11th Edition*. Washington D.C. pp. 16.
- Banerjee, H., Chatterjee, S., Sarkar, S., Gantait, S. and Samanta, S. 2017. Evaluation of rapeseed-mustard cultivars under late sown condition in coastal ecosystem of West Bengal. *J. Appl. Nat. Sci.*, **9**(2): 940 - 949. [\[Cross Ref\]](#)
- Hasan, E.U., Mustafa, H.S.B., Bibi, T. and Mahmood, T. 2014. Genetic variability, correlation and path analysis in advanced lines of rapeseed (*Brassica napus* L.) for yield components. *Cercetări Agronomice în Moldova*, **47**(1): 71–79. [\[Cross Ref\]](#)
- Lodhi, B., Thakral, N.K., Avtar, R. and Singh, A. 2014. Genetic variability, association and path analysis in Indian mustard (*Brassica juncea* L.). *J. Oil. Brass.*, **5**(1): 26–31.
- Mekonnen, T.W., Wakjira, A. and Genet, T. 2014. Correlation and path coefficient analysis among yield component traits of Ethiopian mustard (*Brassica carinata*) at Adet, northwestern, Ethiopia. *J. of Plant Sci.*, **2**(2):89-96.
- Ramanjaneyulu, A.V. and Giri, G. 2007. Correlation and path coefficient analysis in Indian mustard [*Brassica juncea* (L.) Czern & Coss]. *The J. Res. ANGRAU*, **35**: 107–110.
- Ram Avtar, Rani, B., Jattan, M., Manmohan, Kumari, N. and Rani, A. 2016. Genetic diversity analysis among elite gene pool of Indian mustard using SSR markers and phenotypic variations. *The Bioscan*. **11**(4):3035-3044.
- Rout, S., Kerkhi, A. and Chauhan, C. 2018. Character association and path analysis among yield components in indian mustard [*Brassica juncea* (L.) Czern and Coss]. *Int. J. Curr. Microbiol. App. Sci.*, **7**(1): 50-55. [\[Cross Ref\]](#)
- Roy, S.K., Kale, V.A. and Nagnathwar, V.A. 2015. Assessment of genetic variability of rapeseed-mustard germplasm under Terai region of West Bengal. *Electron. J. Plant Breed.*, **6**(4): 1132-1136.
- Sarawgi, A.K., Rastogi, N.K. and Soni, D.K. 1997. Correlation and path analysis in rice accessions from Madhya Pradesh. *Field Crops Res.*, **52**:161-167. [\[Cross Ref\]](#)
- Sandhu, S.K. and Gupta, V.P. 1996. Genetic divergence and correlation studies in *Brassica* species. *Crop Imp.*, **23**:253-256.
- Shekhawat, N., Jadeja, G.C., Singh J. and Shekhawat, R.S. 2014. Character association studies among yield and its component characters in Indian mustard (*Brassica juncea* L. Czern & Coss). *The Bioscan*, **9**(2): 685-688.
- Singh D. 2014. Genetic enhancement of mustard for seed yield & its sustainability. *J. Oilseed Brass.*, **5**(2):180-191.
- Singh, K.H., Shakya, R., Thakur, A.K., Chauhan, D.K. and Chauhan, J.S. 2013. Genetic Diversity in Indian Mustard [*Brassica juncea* (L.) Czern & Coss] as revealed by Agronomic Traits and RAPD Markers. *Natl. Acad. Sci. Lett.*, **36**(4): 419–427. [\[Cross Ref\]](#)
- Singh, M., Srivastava, R.L., Prasad, L. and Dixit, R.K. 2003. Correlation and path analysis in Indian mustard [*Brassica juncea* (L.) Czern & Coss]. *Adv. Plant Sci.*, **16**: 311–315.
- Tuncturk, M. and Ciftci, V. 2007. Relationships between yield and some yield components in rapeseed (*Brassica napus* ssp. *oleifera* L.) cultivars by using correlation and path analysis. *Pak. J. Bot.*, **39**(1): 81-84.

- Vermai, U., Thakral, N.K. and Neeru. 2016. Genetic diversity analysis in Indian mustard [*Brassica juncea* (L.) Czern and Coss]. *IJAMSS*, **6**(2): 2319-3980.
- Vinu, V., Singh, N., Vasudev, S., Yadava, D.K., Kumar, S., Naresh, S., Bhat, S. R. and Prabhu, K.V. 2013. Assessment of genetic diversity in *Brassica juncea* (*Brassicaceae*) genotypes using phenotypic differences and SSR markers. *Rev. Biol. Trop.*, **61**(4): 1919-1934.