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Unrevealing the role of epistasis through Triple Test Cross in Indian mustard

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ABSTRACT: Rapeseed-mustard is premium oilseed crop as far as edible oil availability is considered. Even after intensive improvement programme, still there is need to fill the gap between demand and supply. Estimates of epistasis though plays highly significant role, still always overlooked in almost all the statistical analysis. In present investigation, the epistasis is calculated using triple test cross analysis. The additive × additive [i] type epistasis was found to be significant for days to maturity and plant height. The estimates of non-additive [j+l] epistasis emerged highly significant for all the characters except days to 50% flowering, length of main raceme, siliquae on main raceme, primary branches per plant, number of seeds per siliqua and test weight. Total epistasis showed highly significant estimates for days to maturity, plant height, secondary branches per plant, siliqua length, siliqua density, oil content and seed yield per plant. The [i] type epistasis × blocks exhibited significant estimates only for siliqua length. None of characters showed significant estimates of [j+l] type epistasis x blocks and total epistasis x blocks. The present investigation reflected significant role of epistatic components which should be taken into consideration while suggesting breeding methodology to attain maximum outcome.

Key words: Epistasis, Fixable and non- fixable gene effect, Indian mustard, triple test cross

Rapeseed-mustard is the key oilseed crop and also world's third most important source of edible oil after soybean and palm oil. India is world's third largest producer of rapeseed-mustard after Canada and China. Globally, it contributes almost 11% of production. The average contribution of rapeseed-mustard to the total oilseed production in India was 9.12 million tonne, with its average productivity 1345 (kg/ha) during 2019-20. Though, rapeseed-mustard is placed second in terms of production, after soybean, it is most important primary source of edible oil as ranks first in terms of oil yield among all oilseed crops. The genus *Brassica* is one of 51 genera of Brassiceae family. Mostly cultivated species in India are *B. rapa* var toria, *B. rapa* var. yellow sarson, *B. juncea, B. napus*, *B. carinata and B. nigra* out of which *B. juncea* is the most predominant crop. It is an amphidiploid species (n=18) derived from inter specific crosses between *B. nigra* (n = 9) and *B. rapa* (n = 10). In India, *B. juncea* accounts for more than 90% of the area. Rajasthan, Uttar Pradesh, Gujarat, Madhya Pradesh and Haryana cover more than 80% of acreage under Indian mustard. *B. juncea* is grown in marginal and sub-marginal lands either as pure crop or as a mixed crop with wheat, lentil, chickpea, sugarcane, pea, linseed, etc.

The global demand of edible oils and its products is increasing continuously. The area estimated, production and productivity of rapeseed-mustard during 2011-12 in the world was about 33.05 million ha, 60.57 million tonnes and 1834 kg/ha, respectively. Area production and productivity during 2018-19 has been increased to 36.59 million ha, 72.37 million tonnes and 1980 kg/ha, respectively. Inspite of having all the advancement in the production technology, still a huge gap exists between demand and supply. To meet the ever-increasing demand of edible oil, lots of money has to spend for import of oil. To fill this gap, efforts are required that doesn't leave any stone unturned. From breeder point of view, a deep and better understanding of genetic control of different traits for seed yield should be incorporated while designing any breeding strategy to attain maximum outcome. The genetic phenomena that's effect is considered insignificant must be understood and exploited to its maximum. A phenomenon of epistasis is always present but its effect was considered as negligible. In most of the experiments it is not being calculated and assumed to be absent

as the part of assumption; though it has a great role in deciding the expression of any trait. Epistasis is the interaction between alleles of different genes, i.e., non-allelic interaction, as opposed to dominance, which is interaction between allele of the same gene, called inter allelic or intra-genic interaction (Kearsey and Pooni, 1996). Statistical epistasis describes the deviation that occurs when the combined additive effect of two or more genes does not explain an observed phenotype (Falconer and Mackay, 1996). Because of the complexity of theoretical genetics studies on epistasis, there is a lack of information about the contribution of the epistatic components of genotypic variance when predicting gains from selection. The estimation of epistatic components of genotypic variance is hardly done in genetic studies because of absence of simple methodology. In case of the triple test cross, the high number of generations to be produced and assessed and mainly because only one type of progeny, Half-Sib, Full-Sib or inbred families, is commonly included in the experiments (Viana, 2005). Triple test cross (TTC) design developed by Kearsey and Jinks (1968) is an extension of North Carolina Design III of Comstock & Robinson (1952) that is applicable to any population irrespective of its mating system and its gene and genotype frequencies. In the absence of epistasis, TTC also provides unbiased estimates of additive (D) and dominance (H) components of genetic variation, degree of dominance [(H/D)1/2] as well as the direction of dominance (rs.d) with high degree of precision. Ketata *et al*. (1976) suggested a similar model of TTC where testers L1, L2 and L3 (L3=L1 x L2) were crossed to a number of varieties instead of F_2 individuals as proposed by Kerasy and Jinks (1968). Epistasis might play an important role as the genetic basis of Heterosis. Additive and dominance effect may have a greater influence on variation in grain yield and other agronomic character, information on nonallelic interactions in winter wheat would be value to wheat breeders. The nonallelic interactions might inflate the average degree of dominance by 10 to 25%. The preponderance of epistasis effect in the inheritance of quantitative trait in crops was recently reported by many geneticists (Bnejdi and El Gazzah, 2008; Bnejdi *et al*., 2009; Bnejdi and El-Gazzah, 2010a; Devi *et al*., 2018; Meena *et al*., 2019). Epistasis can have an important influence on a number of evolutionary phenomena, including the genetic divergence between species. Therefore, the present investigation was carried out to unreveal the role of epistasis in controlling the seed yield and its component traits in Indian mustard (*Brassica juncea* L.).

MATERIALS AND METHODS

Eleven diverse mustard varieties/strains (Divya, NDRE-4, Kanti, NPJ-112, PRE-10-19, Maya, PR-20, Krishna, Albeli, RB-57, PRB-08-5) were selected as lines and PRE-10-15, RGN-73 and PRE-10-15×RGN-73 was crossed to lines as testers to produce crosses for triple test crosses analysis to estimate epistasis. A set of experimental materials consisting of 14 parents, 33 crosses were grown in Randomized Block Design (RBD) with three replications in single environments. Parents and crosses were sown in single row of 3m long, spaced at 30cm apart. The distance of 10 cm between the plants within row was maintained by thining after 15-20 days of sowing. To avoid border effect, experimental plots were surrounded by one non-experimental row of standard variety Kranti treated as standard variety (check) in experiments. All the recommended package of practices was followed to raise good crop. Observations were recorded on 13 morphological traits namely days to 50% flowering, days to maturity, plant height, length of main raceme, siliqua on main raceme, siliqua density, primary branches per plant, secondary branches per plant, siliqua length, seeds per siliqua, 1000-seed weight, seed yield per plant and oil content as per the standard protocol. Data recorded on different parameters were subjected to suitable statistical procedures as given by Ketata *et al.*, 1976 for triple test cross analysis and test of epistasis.

RESULTS AND DISCUSSION

Analysis of variance for Triple Test Cross analysis: Highly significant differences were observed among treatments for all the characters (Table 1). Highly significant variation was observed for parents for days to 50% flowering, days to maturity, plant height,

siliqua length, seeds per siliqua, siliqua density, seed yield per plant, oil content and test weight. The differences due to lines were significant for days to 50% flowering, days to maturity, plant height, siliqua length, siliqua density, oil content, seed yield per plant and test weight whereas in case of testers significant differences were recorded for days to maturity, plant height and seed yield per plant which indicated worth of testers. Significant variation due to Lines *vs* testers was observed only for days to maturity. The crosses exhibited significant difference for all the characters except seeds per siliqua which indicated presence of sufficient difference between lines and testers. The variances due to parents *vs* F_1 were found significant for all the characters excepts days to maturity, oil content and test weight which further indicated expression of non fixable gene effect for characters under study. Various biometrical methods have been used in different crops in the past to estimate various types of gene effects. In most of the designs used, it is assumed that non-allelic interactions are absent, whereas the fact is often contrary to the assumption. Information on genetics, especially on epistatic gene effects for improvement of characters in rapeseed is extremely important. A survey of pertinent literature on rapeseed and mustard revealed the importance of epistasis in the expression of yield and component traits in a number of studies (Joarder and Eunus, 1970; Zuberi *et al.*, 1972; Patnaik and Murty, 1978; Chaudhary and Sharma, 1982; Sachan and Singh, 1986; Tripathi, 2005; Upadhyaya and Kumar, 2014; Mall and Bhajan, 2015, Devi *et al.,* 2018, Meena *et al.,* 2019, Jabeen and Chadha, 2021). Among various mating designs available to study the genetic variability, the triple test cross is the most recent one. It provides precise estimates of various genetic parameters together with the availability of test for epistasis which is not envisaged in other multiple mating designs.

Table 1: Analysis variance for Triple Test Cross analysis

Source of Variations	d.f	Days to flowering	Days to maturity	Plant height	Length of main raceme	Siliqua on main raceme	Primary branches	Secondary branches	Siliqua length	Seeds per siliqua	Siliqua density	Seed yield	Oil content	Test weight
Replications	2.00	0.69	0.30	19.12	36.11	29.74	1.37	79.02	0.80	1.20	0.01	31.35	28.30	1.67
Treatments	46.00	19.15**	78.31**	1026.49**	83.01**	31.56*	2.96**	63.36**	0.27**	2.24*	0.02**	10.05**	19.72**	0.37**
Parents	13.00	15.30**	105.22**	1089.33**	44.78	15.89	1.86	13.59	0.31**	2.80*	0.02**	5.52**	25.04**	0.44**
Lines	10.00	19.56**	116.79**	1125.34**	47.66	12.90	1.87	14.32	0.37**	2.27	0.02**	6.00**	28.86**	0.53**
Testers	2.00	1.33	90.78**	1447.10**	0.86	6.59	1.33	16.70	0.17	4.23	0.004	5.44**	11.30	0.00
L vs T	1.00	0.65	18.47**	13.73	103.80	64.29	2.80	0.07	0.00	5.18	0.007	0.84	14.36	0.33
Crosses	32.00	20.96**	69.82**	908.84**	75.21*	28.04*	3.19**	77.43**	0.24**	1.76	0.02**	11.76**	17.76**	0.36**
Parent vs crosses	1.00	11.35*	0.18	3974.38**	829.69**	348.11**	9.99**	259.77**	0.58**	10.62**	0.01*	14.45**	13.11	0.05
Error	92.00	1.70	1.11	76.30	42.45	17.84	1.44	14.14	0.07	1.37	0.001	1.12	4.82	0.10
Total	140.00	7.42	26.46	387.69	55.68	22.52	1.94	31.24	0.14	1.66	0.01	4.48	10.05	0.21

Test of Epistasis

The mean squares due to epistasis for 13 seed yield and component characters are given in Table 2. Total epistasis showed highly significant estimates for days to maturity, plant height, secondary branches per plant, siliqua length, siliqua density, oil content and seeds yield per plant. Further fractionation of total epistasis in to [i] type and [i+j] types showed that the variation due to [i] type epistasis was found to be significant for days to maturity and plant height which insures preponderance of additive gene action. The estimates of [j+l] type epistasis emerged highly significant for days to maturity, plant height Siliqua length, Secondary branches, seeds per siliqua, Siliqua density, Seed yield per plant and oil content wherein the role of non-fixable gene action is evident. The [i] type epistasis × blocks exhibited significant estimates only for siliqua length. The interaction of total, i type and j + l types of epistasis

Table 2: ANOVA for test of epistasis for seed yield and its component characters

Characters	Mean sum of squares					
d.f	[i] type epistasis	[j+l] type epistasis	Total epistasis	[i] type epistasis × block	[j+l] type epistasis × block	Total epistasis × block
	1	10	11	2	20	22
Days to flowering	0.03	10.54	9.60	10.39	12.82	12.60
Days to maturity	458.45*	78.38***	112.93**	11.54	6.48	6.93
Plant height	6392.62*	1823.14**	2338.55**	90.54	412.24	382.99
Length of main raceme	28.00	296.09	271.72	342.30	330.40	331.48
Siliqua on main raceme	118.37	181.81	176.04	312.73	80.49	101.61
Primary branches	65.24	12.02	16.86	7.91	7.91	7.91
Secondary branches	95.23	233.75*	221.16*	66.18	83.90	82.29
Siliqua length	2.017	0.79**	0.90**	1.03*	0.16	0.24
Seeds per siliqua	0.91	11.76	.78	0.21	8.84	8.05
Siliqua density	0.06	0.132**	0.12**	0.01	0.01	0.01
Seed yield	79.34	65.78**	67.01**	4.60	9.85	9.37
Oil content	47.47	66.28*	64.57**	3.82	21.76	20.12
Test weight	3.45	0.39	0.66	0.64	0.91	0.89

*, ** significant at 5% and 1% probability levels, respectively

with replications were non-significant which indicated that these interactions were not sensitive to the environments (replications).

Cockerham (1961) stated that the relative merits of current methods of selection with regard to epistatic gene action are not known. Nevertheless, it is argued that standard hybridization and selection could take advantage of epistasis if it is of additive type (additive × additive). The epistasis components involving dominance (additive × dominance, additive × additive × dominance etc.) are not fixable by selection under self fertilization (Dickerson, 1963) and therefore, would not be favourable for developing pure line cultivars. However, these may be used through the development of hybrids and/or use of appropriate recurrent selection procedure. In the development of pure line selection is delayed until virtual homozygosity is attained, since only additive types of epistasis are present in the pure lines (Matzinger, 1963). These findings, therefore, indicate that epistasis is an integral part of genetic system and plays an important role in the inheritance of characters. By ignoring the presence of epistasis, one would not only loose some important genetic information but the estimates of additive and dominance components would also be biased. The detection and estimation of epistasis would enable a breeder to determine the genetic cause of heterosis with greater precision. Ketata *et al*. (1976) stated that epistasis is determined by the nature of genotypes and to some degree by the number of lines used. Pooni *et al*. (1980) highlighted that the best possible experimental size necessary to detect epistasis through TTC depends mostly on gene distribution in the tester parents, therefore several lines and diverse testers (L1 and L2) should be employed to detect epistasis.

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