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GENETIC DIVERSITY REVEALED BY MORPHOLOGICAL DESCRIPTORS AMONG YELLOW SARSON GERMPLASM UNDER TIMELY AND LATE SOWN CONDITIONS

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ABSTRACT

Thirty-one local germplasm lines of *Brassica rapa* var. yellow sarson, collected from eastern parts of Uttar Pradesh (India) were evaluated along with two commercial varieties as checks in two environmental conditions (timely sown E_1 and late sown E_2 conditions). Data collected on 14 morphological characters was subjected to D^2 statistics. The 31 germplasm lines were classified into 8 and 12 clusters under E_1 and E_2, respectively. In E_1, cluster I had a maximum of 15 genotypes while cluster III, VII and VIII had only one genotype each, in E_2, cluster III had maximum of 15 genotypes whereas cluster IV, V, VII, VIII, X, XI, and XII had only one genotype each. Likewise, the inter-cluster average D^2-value was maximum (26.81) between cluster III and VIII and minimum between cluster V and I (2.80). Intra-cluster average D^2 values ranged from 0.00 to 4.63. In late sown condition, the inter-cluster distance was maximum between IV and II (51.93) and minimum in cluster VI and IX (4.965). Intra-cluster average D^2 value ranged from 0.0 to 4.594. The genotypes which occupied distant clusters can be used in hybridization programme as parents to recover more heterotic hybrids. Secondary branches plant^{-1}, glucosinolate content, seed yield, seeds siliqua^{-1}, primary branches plant^{-1} and siliqua length contributed maximum towards total divergence in timely sown condition whereas secondary branches plant^{-1}, seed yield, primary branches plant^{-1}, glucosinolate content, and main raceme length were the major contributors in late sown conditions.

Key words: *Brassica rapa*, cluster analysis, D^2-statistics, genetic divergence

INTRODUCTION

Rapeseed-mustard crop is the second most important oilseed crop in India after soybean. It accounts for 20- 22% of the total oilseeds produced in the country (FAO, 2009). The genus *Brassica* is one of 51 genera in tribe *Brassiceae* belonging to the crucifer family which has great economic value and plays a major role in feeding the world population. It provides over 16% of the world's edible oil and also gives nutritious vegetables and condiments to animal fodders (Ahmad *et al*., 2007). In India, rapeseed-mustard is cultivated on 6.70 million ha with net production of 7.96 million tonnes and average yield of 1188 kg ha^{-1} during 2013-2014 (Anonymous, 2014). These crops mainly belong to 4 species *viz.*, *Brassica juncea, B. napus, B. rapa* and *B. carinata* of tribe *Brassiceae* within the family Cruciferae (*Brassicaceae*), are grown worldwide. B. *rapa* has three ecotypes; *B. rapa* var. yellow sarson, *B. rapa* var. toria and *B. rapa* var. brown sarson. Amongst the rapeseed-

mustard group *B. rapa* var. yellow sasron is favoured for high oil recovery and quality. Despite all the efforts made by scientists across the country the productivity of this crop has been low while the demand for oil has escalated with increase in population and economic status that warrants developing innovative strategy to enhance the yield of this important oilseed crop.

Improvement in crop plants depends upon the magnitude of genetic variability in different quantitative characters (Gupta *et al.*, 2015). A breeding programme may rightly be formulated on the basis of available information on the extent of genetic diversity (Chauhan *et al.*, 2008; Singh *et al*., 2013). Oilseed *Brassicas* represent a rich diversity, which are cultivated in 23 states and union territories (Misra and Kumar, 2008; Misra *et al.*, 2010). However, much of this diversity is concentrated in the Indo-Gangetic plains and the sub-mountain Himalayas. Genetic diversity plays an important role in plant breeding because a hybrid between lines of diverse origin generally display greater heterosis than those between closely related strains (Singh, 1983; Naznin *et al.*, 2015) which permit the selection of genetically divergent plants to obtain the desirable recombination of segregating generation. Multivariate analysis is a useful tool in quantifying the degree of divergence between biological populations at genotypic level and to assess the relative contribution of different components to the total divergence both at intra-cluster and inter-cluster levels (Jatasra and Parada, 1978; Zahan *et al.*, 2008). Therefore, the present study was undertaken to assess to assess the extent of variability in collected germplasm accession and to identify divergent parents for hybridization program, which would provide superior transgressive segregants from collected germplasm.

MATERIALS AND METHODS

The experiment was conducted at the Norman E. Borlaug Crop Research Centre, Pantnagar, Uttranchal (India) during the *rabi* (winter) season of the year 2012-13. The experimental material comprised of thirty-one germplasm lines of *Brassica rapa* var. yellow sarson collected from eastern parts of Uttar Pradesh (India) with two check varieties namely PPS-1 and B-9. The experiment was laid out in a randomized block design with three replications in two environmental conditions *viz.*, timely sown condition (October 2, 2012) and late sown condition (November 3, 2012). Each plot was of three rows of 3 m length, 30 cm apart and spacing between plants was maintained at 10 cm by thinning. Data were recorded on five randomly selected plants for twelve different characters *viz.*, plant height, days to maturity, days to 50% flowering, number of primary branches plant^{-1}, number of secondary branches plant^{-1}, number of siliquae plant^{-1}, number of seeds siliqua^{-1}, 1000-seed weight, oil content^{-1} and seed yield plant^{-1}, in each genotype and replication. Glucosinolate content was analyzed by sodium tetrachloropalladate [$Na_2(PdCl_4)$] with the help of ELISA reader (BioTek ELx800 using software Gen5.) given by Thies (1982) and the oil content in the sample was determined as per the Soxhlet method (destructive approach). The data was analyzed following Mahalanobis's D^2- statistics (Mahalanobis, 1936) for assessment of genetic divergence among the genotypes (Rao, 1952; Singh and Chaudhary, 1985). Tocher's method was used for clustering D^2 values as per Rao (1952) while intra-cluster and inter-cluster distances were calculated using the formula of Singh and Choudhary (1977).

RESULTS AND DISCUSSION

The analysis of variance revealed highly significant differences among all the 31 genotypes for all the 14 characters studied in two environmental conditions *viz.*, timely (E_1) and late sown (E_2) conditions (Table 1). This suggested the presence of significantly high magnitude of genetic variability among the

Table 1: Analysis of variance for various sarson characters under timely sown [E_1] and late sown [E_2] environmental conditions

Characters	Source of variation for E_1 environment					Source of variation E_2 environment				
	Replica-tions (2)	Treatments (30)	Error (60)	$CD_{0.05}$	CV%	Replica-tions (2)	Treatments (30)	Error (60)	$CD_{0.05}$	CV%
50% flowering	1.55	274.14*	6.72	4.23	5.59	34.21	33.97**	12.09	5.68	7.54
Maturity	3.62	273.23**	3.51	3.06	1.72	2.90	168.28**	3.11	2.88	1.46
Plant height (cm)	9.76	261.37 **	55.15	12.13	5.99	95.01	272.21**	20.67	7.43	5.27
Length of main raceme (cm)	11.91	105.85**	16.59	6.65	7.88	41.26	348.63**	23.36	7.89	12.01
Siliqua on main raceme (No.)	19.73	87.52**	12.87	5.86	9.66	18.93	55.86**	8.58	4.78	13.40
Siliqua density	0.00	.00.14**	0.29	0.11	9.60	0.03	0.02**	0.007	0.14	15.22
Primary branches (No. plant^{-1})	17.32	2.30	1.88	2.24	15.45	0.05	4.28**	0.51	1.17	11.53
Secondary branches (No. plant^{-1})	0.18	10.20**	0.13	0.60	2.03	0.02	1.06**	0.03	0.29	20.82
Siliqua length (cm)	1.31	0.65**	0.18	0.69	7.28	0.32	0.41**	0.10	0.52	8.19
No. of seeds (siliqua^{-1})	108.9	91.82**	16.39	6.61	11.97	36.26	28.06**	3.27	2.95	6.21
Test weight (g)	0.36	0.67**	0.060	0.40	6.01	0. 70	0.36*	0.18	0.69	14.67
Yield plant^{-1} (g)	23.78	39.91**	4.86	3.60	17.06	1.88	63.69**	1.82	2.20	10.85
Glucosinolate content (µmol g^{-1})	2.24	4906.29**	34.56	9.60	4.15	24.46	4600.23**	27.22	8.52	3.63
Oil content (%)	1.44	1.69	1.49	1.99	2.75	1.18	1.57	1.12	1.73	2.49

*,** for 5 and 1% level of significance

collected local germplasm. The mean, range, coefficient of variation, heritability and genetic advance for all traits for both the environments revealed maximum variability in the number of secondary branches plant^{-1}, followed by seed yield plant^{-1} and glucosinolate content obtained under both the environmental conditions (Table 2 and 3).

D^2 analysis grouped the germplasm into 8 clusters in timely sown condition (Table 4). Cluster I comprised of 15 genotypes, followed by cluster II, V, IV and VI which consist of 4, 4, 3 and 2 genotypes, respectively. Cluster III, VII and VIII had only one genotype in each cluster. The inter-cluster distances were larger than intra-cluster distances in most of the cases suggesting wider genetic diversity among the genotypes (Table 5). The maximum inter-cluster average D^2-value was observed between cluster III and VIII (26.812), followed by between cluster VII and VIII (24.901),

Table 2: Range, general mean (GM), standard error mean (SE_m) and variability in yellow sarson under timely sown (E_1) condition

Characters	Range	GM	SE_m±	PCV	GCV	ECV	h^2	G_A
50% flowering	33.67-62.67	46.39	1.50	21.11	20.35	5.59	93.00	18.76
Maturity	99.0-129.33	109.28	1.08	8.84	8.68	1.72	96.24	19.16
Plant height (cm)	108.4-151.3	124.00	4.29	8.98	6.69	5.99	55.50	12.72
Main raceme length (cm)	36.87-69.47	51.67	2.35	13.18	10.56	7.88	64.21	9.00
Siliqua on main raceme (No.)	28.80-48.53	37.15	2.07	16.54	13.43	9.66	65.91	8.34
Siliqua density	0.59-0.88	0.72	0.04	12.36	7.79	9.60	39.67	0.07
Primary branches plant^{-1} (No.)	3.76-10.80	8.63	0.79	16.66	6.23	15.45	14.00	0.43
Secondary branches plant^{-1} (No.)	0.00-9.93	2.03	0.21	92.00	90.20	18.07	96.14	3.70
Siliqua length (cm)	4.53-7.13	5.84	0.25	9.95	6.78	7.28	46.44	0.56
Seeds siliqua^{-1} (No.)	25.73-45.69	33.82	2.34	19.05	14.83	11.97	60.54	8.04
Test weight (g)	2.77-4.77	4.10	0.14	12.49	10.96	6.01	76.90	0.81
Yield plant^{-1} (g)	6.22-19.92	12.92	1.27	31.48	26.45	17.06	70.63	5.92
Glucosinolate (µmol g^{-1})	69.6-249.9	141.70	3.39	28.73	28.43	4.15	97.92	82.14
Oil content (%)	43.34-45.53	44.37	0.70	2.81	0.58	2.75	4.32	0.11

Table 3: Range, general mean (GM), standard error mean (SEm) and variability parameters in yellow sarson in late sown {E_2} condition

Characters	Range	GM	SEm±	PCV	GCV	ECV	h2	GA
50% Flowering	40.67-54.33	46.14	2.00	9.54	5.85	7.54	37.62	3.41
Maturity	103-131.3	120.70	1.02	6.32	6.15	1.46	94.65	14.87
Plant height (cm)	73.27-116.1	86.23	2.62	11.86	10.62	5.27	80.23	16.90
Main raceme length (cm)	27.93-84.20	40.24	2.79	28.53	25.87	12.01	82.28	19.46
Siliqua on main raceme (No.)	16.47-35.33	21.86	1.69	22.57	18.16	13.40	64.76	6.58
Siliqua density	0.42-0.78	0.56	0.05	19.67	12.45	15.22	40.10	0.09
Primary branches plant^{-1} (No.)	0.80-7.87	6.20	0 .41	21.45	18.09	11.53	71.13	1.95
Secondary branches plant^{-1} (No.)	0.00-1.80	0.84	0.10	72.54	69.49	20.82	91.76	1.16
Siliqua length (cm)	3.21-4.73	3.91	0.18	11.61	8.23	8.19	50.27	0.47
Seeds siliqua^{-1} (No.)	18.60-33.07	29.10	1.04	11.67	9.88	6.21	71.63	5.01
Test weight (g)	1.57-3.59	2.87	0.24	16.98	8.55	14.67	25.33	0.25
Yield plant^{-1} (g)	3.63-23.56	12.43	0.78	38.10	36.53	10.85	91.89	8.97
Glucosinolate (µmol g^{-1})	72.9-240.7	143.70	3.01	27.41	27.17	3.63	98.24	79.72
Oil content (%)	41.31-43.76	42.54	0.61	2.65	0.91	2.49	11.66	0.27

PCV = Phenotypic coefficient of variation, GCV = Genotypic coefficient of variation, ECV = Environmental coefficient of variation and h^2 = Heritability, G_A = Genetic advance

IV and VIII (20.17). Cluster III and VIII showed maximum distance and the genotypes in these clusters were more diverse so might serve as potential parents for future hybridization programme in order to recover highly heterotic cross combinations. The minimum inter-cluster distance was observed between cluster V and I (2.801) revealing a close relationship among the genotypes of these cluster. The highest intra-cluster distance was in cluster I (4.631), which had 15 genotypes, followed by cluster IV (4.06) with 3 genotypes and cluster II (2.359) and cluster V (2.069) with 4 genotypes each. Some clusters (III, VII and VIII) showed zero intra-cluster value because of a single genotype in cluster. Gangapur *et al.* (2010) used D^2 statistic for genetic analysis in Indian mustard and grouped 46 genotypes into 7 clusters in both (protected and unprotected) conditions.

Table 4: Clustering patterns of thirty one genotypes on the basis of D^2 values in E_1 condition

Cluster No.	Genotypes included	No. of genotypes
I	PYSC-11-1, PYSC-11-3, PYSC-11-5, PYSC-11-6, PYSC-11-11, PYSC-11-13, PYSC-11-20, PYSC-11-22, PYSC-11-23, PYSC-11-25, PYSC-11-27, PYSC-11-30, PYSC-11-36, PYSC-11-39, PYSC-11-41	15
II	PYSC-11-2, PYSC-11-16, PYSC-11-18, B-9	4
III	PYSC-11-4	1
IV	PYSC-11-7, PYSC-11-48, PPS-1	3
V	PYSC-11-15, PYSC-11-19, PYSC-11-29, PYSC-11-34	4
VI	PYSC-11-17, PYSC-11-32	2
VII	PYSC-11-24	1
VIII	PYSC-11-35	1

Table 5: Average inter- and intra-cluster D^2 values in E_1

Clusters	I	II	III	IV	V	VI	VII	VIII
I	4.631	6.472	10.929	5.870	2.801	5.881	10.464	12.694
II		2.359	16.614	11.136	7.937	11.033	19.423	17.269
III			0.000	11.906	11.577	14.751	17.289	26.812
IV				4.064	8.333	9.828	10.596	20.170
V					2.069	3.364	12.412	13.345
VI						1.619	11.867	10.999
VII							0.000	24.901
VIII								0.000

Mahmud *et al.* (2011) used D^2 analysis in *B. rapa* and grouped genotypes into 6 clusters. Singh *et al.* (2013) and Lodhi *et al.* (2013) grouped Indian mustard genotypes into 6 and 9 different clusters.

Under late sown conditions the experimental material was grouped into 12 clusters (Table 6). Cluster III comprised of the highest number of genotypes (15) followed by cluster VI, I and IX comprised of 4, 2 and 2 genotypes, respectively. Cluster II, IV, V, VII, VIII, X, XI and XII had one genotype. The inter-cluster distance was maximum between IV and II (51.93), followed by XI and IV (48.096), X and V (45.464) that revealed that the genotype in these groups were highly diverse and can be

Table 6: Clustering patterns of thirty one genotypes on the basis of D^2 values in E_2 condition

Cluster No.	Genotypes included	No. of genotypes
I	PYSC-11-1, PYSC-11-34	2
II	PYSC-11-2	1
III	PYSC-11-3, PYSC-11-6, PYSC-11-11, PYSC-11-13, PYSC-11-15, PYSC-11-16, PYSC-11-18, PYSC-11-23, PYSC-11-25, PYSC-11-27, PYSC-11-29, PYSC-11-30, PYSC-11-36, PYSC-11-39, PYSC-11-48	15
IV	PYSC-11-4	1
V	PYSC-11-5	1
VI	PYSC-11-7, PYSC-11-20, PYSC-11-32, B-9	4
VII	PYSC-11-17	1
VIII	PYSC-11-19	1
IX	PYSC-11-22, PPS-1	2
X	PYSC-11-24	1
XI	PYSC-11-35	1
XII	PYSC-11-41	1

used for hybridization purpose. Those clusters which had noticed minimum distance were VI and IX (4.965) followed by III and IX (5.037), III and VI (6.087), respectively, considered to be less diverse (Table 7). The intra-cluster distance varied from 0.00 to 4.594, while maximum for cluster VI (4.594) comprised of 4 genotypes followed by cluster III (4.293) including 15 genotypes, cluster I (2.785) and cluster IX (2.709) that comprised only two genotypes. The minimum intra-cluster distance observed in cluster II, IV, V, VII, VIII, X, XI and XII consisted of only single genotype so no intra-cluster distance was observed. These results revealed that the genotypes in cluster VI were distantly related; on the other hand, the genotypes in cluster I and IX were closely related. Patel and Patel (2015) used D^2 statistic for seed yield and their component trits in Indian mustard and genotypes were characterized 5 clusters. In both the environmental condition some genotypes (PYSC-11-4, PYSC-11-24 and PYSC-11-35) showed single genotype in cluster which revealed that these genotypes are highly diverse. The clustering pattern of genotypes revealed that the varieties/ lines originating from the same places did not form a single cluster because of direct selection pressure. The genotypes of distant cluster could be used for further hybridization program. The inter-cluster and intra-cluster distances were also depicted in Fig. 1 and 2 which shows the distances between the genotypes presents in clusters.

Table 7: Average inter and intra-cluster D^2 values in E_2

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	2.785	20.650	6.407	28.768	16.719	12.038	14.271	11.230	13.162	23.241	27.981
II		0.000	15.647	51.930	32.538	19.343	26.572	26.976	24.878	18.065	22.466
III			4.293	19.901	19.156	6.087	13.381	12.089	5.037	15.455	24.906
IV				0.000	21.323	26.214	44.221	44.258	22.704	34.125	48.096
V					0.000	28.451	30.236	29.680	27.008	45.464	34.654
VI						4.594	11.375	13.987	4.965	12.061	27.300
VII							0.000	12.081	16.052	23.701	27.757
VIII								0.000	12.411	26.580	27.420
IX									2.709	21.982	30.009
X										0.000	26.517
XI											0.000

Table 8: Contribution of different characters towards divergence under E_1 and E_2 environments

S. No.	Characters	Contribution (%)	
		E_1	E_2
1	Days to 50%flowering	4.77	3.04
2	Days to maturity	2.71	2.33
3	Plant Height (cm)	1.35	4.80
4	Main raceme length (cm)	1.70	9.22
5	Siliqua on main raceme (No.)	2.94	7.63
6	Siliqua density	1.97	6.28
7	Primary branches (No. plant^{-1})	5.84	11.15
8	Secondary branches (No. plant^{-1})	45.72	16.33
9	Siliqua length (cm)	4.47	4.21
10	Seeds (No. siliqua^{-1})	6.06	4.77
11	Test weight (g)	3.84	6.27
12	Yield (g plant^{-1})	6.99	12.65
13	Glucosinolate (µmol g^{-1})	11.13	10.67
14	Oil content (%)	0.50	0.66

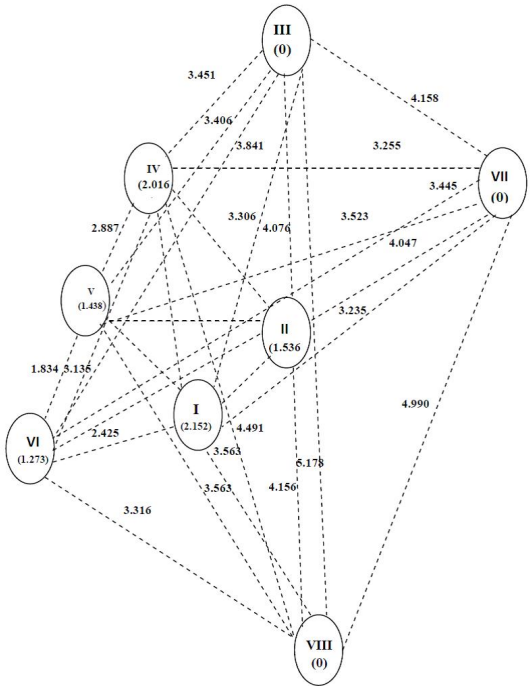

Fig. 1: Diagrammatic representation of intra- and inter-cluster distances among the 31 yellow sarson germplasm in E_1

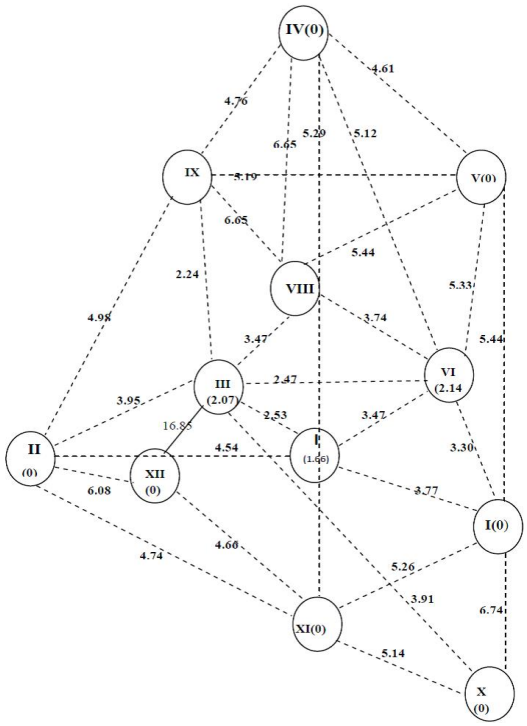

Fig. 2: Diagrammatic representation of intra- and inter-cluster distances among the 31 yellow sarson germplasm in E_2

Contribution of characters towards divergence of the genotypes

The relative contribution of different characters towards the expression of genetic divergence (Table 8) was calculated by following the methods suggested by Singh and Chaudary (1977). The maximum contribution towards divergence was given by the number of secondary branches plant^{-1} in both E_1 (45.7%) and E_2 (16.3%) conditions (Gangapur *et al*., 2010). The lowest contribution was made by oil content in both the environments. The plant height contributed least in E_1 (1.35%) but high in E_2 (4.8%). The number of primary branches plant^{-1} and yield plant^{-1} showed moderate divergence under both the conditions. The crosses involving parents belonging to maximum divergent clusters are expected to manifest maximum heterosis and also wide genetic variability. Keeping this in view, it appears that crosses between genotypes belonging to cluster III and VIII, IV and VIII would give a high manifestation of heterosis in E_1 as well as in E_2 condition cluster IV and II, XI and IV and cluster X and V will give a wide spectrum of genetic variation in segregating generations. Such studies are highly effective in identification and selection of parents for the hybridization programme. A higher level of heterosis and a maximum number of transgressive segregants can be expected from the crosses between genetically distant parents (Lodhi *et al.*, 2013).

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