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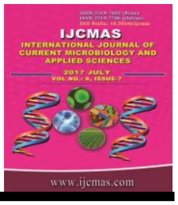

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Studies on Genetic Diversity among Various Genotypes of *Brassica napus* L. Using Morphological Markers

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ABSTRACT

Keywords

Genetic diversity, *Brassica napus*, Correlation coefficients, Path analysis, Cluster analysis.

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The seed material of 18 genotypes of *Brassica napus* L. was procured from different institutes. In order to check the authenticity of the work four other genotypes of different *Brassica* species were also used. Genetic diversity of these genotypes was assessed using morphological traits. The characters days to maturity, plant height, number of primary branches/plant, number of seeds/siliqua, number of siliqua/plant and seed yield /plant showed higher influence of environment whereas, siliqua length and 1000-seed weight showed the least. Days to maturity and days to 50% flowering exhibited the highest heritability. The significant positive correlation with seed yield/plant was found in plant height, number of primary branches/plant, number of siliqua on main raceme, 1000-seed weight, oil content, days to 50% flowering and days to maturity. Path coefficient analysis showed that the plant height had maximum positive direct effect on seed yield followed by 1000-seed weight and siliqua length. Plant height, number of primary branches/plant and number of siliqua on main raceme were the most important contributors to seed yield/plant which could be taken consideration in future selection program. Significant genetic variability was obtained among the selected 22 genotypes through dendogram analysis the genotypes *viz.*, AKGS-3, EC552608 were more diverse from the rest of the *Brassica napus* L. sps. So, the genotypes AKGS-3 and EC55208 should be used to exploit heterosis in hybridization programme with the other *Brassica napus* genotypes considered in the study.

Introduction

Rapeseed and mustard are major rabi oil seed crops of India. Oilseed rape (*Brassica napus* L.) is the most important source of vegetable oil and the second most important oilseed crop in the international oilseed market after soybean (Hasan *et al.*, 2006). *Brassica napus* is an amphidiploid (AACC genome, 2n = 38) and is believed to have arisen by inter-specific hybridization between diploid *Brassica rapa L.* (AA genome, 2n = 20) and *Brassica oleracea* L. (CC genome, 2n = 18). Because of intensive breeding processes *Brassica napus* L. has a relatively narrow genetic diversity in current germplasm. In order to estimate the genetic variation among the diverse group of important crops in *Brassica* genus it have been used a variety of morphological and molecular markers.

The aim of this study was to estimate the genetic diversity among some oilseed rape cultivars based on morphological characterization. For this purpose, 22 oilseed cultivars were analyzed and the results of genetic distances were estimated.

Materials and Methods

Plant material

The plant material for this study comprised 22 genotypes of *Brassica* (Fig. 1). The plants were sown in the field in the year 2013-2014 in order to obtain the morphological data.

Each treatment was sown in 3 rows of 5 m length. The recommended dose of fertilizer was given and also the recommended Plant Protection measures were adapted for raising a good crop. Genotypes with their pedigree are shown in table 1.

Experimental observations

Five plants were randomly selected from each treatment in each replication for recording the observations. These plants were tagged and detailed observations were recorded on all the selected traits:

Data analysis

Statistical analysis such as correlation, coefficients of variability, heritability, genetic advance and path analysis was done using viva Statistical Analysis System (SAS) Software version 9.3.

Analysis of variance

The analysis of variance for various characters studied in experiments was carried out according to the analysis of variance for R.B.D.

Where,
$\sigma^2 e_{ii}$ and $\sigma^2 g_{iii}$ are environmental and genotypic variances of i^{th} character, respectively.

Components of variance

This was calculated by the formula suggested by Burton and De Vane (1953).

Phenotypic variance

$$(\sigma_p^2)=(\sigma_g^2)+(\sigma_e^2)$$

Where,
σ_e^2 = error variances= E.M.S.

Genotypic variance (σ_g^2)

$$\sigma_g^2=\frac{M_v-M_e}{r}$$

Where,
M_v = treatment mean squares
M_e = error mean squares
r = no. of replications

Coefficients of variability

This was calculated by the formula suggested by Burton and De Vane (1953).

Phenotypic coefficient of variability (P.C.V):

$$\text{P.C.V (\%)} = \sqrt{\frac{\sigma_p^2}{\bar{X}}} \times 100$$

Genotypic coefficients of variability (G.C.V):

$$\text{G.C.V (\%)} = \sqrt{\frac{\sigma_g^2}{\bar{X}}} \times 100$$

Where, $\bar{X}$ is the general mean of the character.

Heritability

Heritability was calculated according to Singh and Ceccarelli (1996).

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2+\sigma_e^2} \text{ or } \frac{\sigma_g^2}{\sigma_p^2}$$

Genetic advance

Genetic advance was also estimated according to Allard (1960).

GA = (K) (h^2) ($\sqrt{\sigma p^2}$)

Where, **'k'** is selection differential and at 5% the K value was 2.06.

Genetic advance as per cent of mean (G.A.%):

$$G.A.\% = \frac{genetic\ advance}{\bar{X}} \times 100$$

Correlation coefficients

The simple correlation coefficients between different characters at genotypic and phenotypic level were worked out between characters as suggested by al- Jibouri *et al.*, (1958).

Phenotypic correlation coefficients (r_p)

$$r_p = \frac{Cov.XY(p)}{\sqrt{Var.X(p).Var.Y(p)}}$$

Genotypic correlation coefficients(r_g)

$$r_g = \frac{Cov.XY(g)}{\sqrt{Var.X(g).Var.Y(g)}}$$

Where, Cor. XY(p) and cov. XY (g) denote phenotypic and genotypic covariances between character X and Y, respectively. Var. X (p) and var. X(g) denote variance for characters X and Y, at phenotypic and genotypic levels, respectively. The significance of different correlation coefficients was tested against (v-2) degrees of freedom at 5% and 1%, where v is the no. of varieties on which the observations were recorded.

Path coefficient analysis

The path coefficient was done following the procedure outlined by Dewey and Lu (1959) using genotypic correlation of 'cause' with 'effects' was calculated by following simultaneous equations:

rmp = pmp + rmn pnp + rmo pop …1
rnp = rnm pmp + pnp + rno pop …2
rop = romp mp+ ron pnp + pop …3

Where, Pmp, Pnp, Pop are direct affects of m, n and o on cause P, and rmp, pnp, rmo, Pop… are indirect affects on cause. These simultaneous equations are solved by using matrix method expressed below:

rmp = rmp rmn rmo Pmp
rnp = rnm rnn rno Pnp
rop = rom ron roo Pop

Or A = B.C. Here, A and B vectors are known. For calculation of C vectors the formula used is:

C = B^{-1}, A

Here, B^{-1} is the inverse matrix of B vector. Pivotal condensation method was used for matrix inversion.

Results and Discussion

Genetic variability

Generally phenotypic coefficients of variability were higher than genotypic coefficient of variability which indicates that environment plays a considerable role in the expression of these traits. The maximum phenotypic and genotypic coefficient of variability was observed for number of seeds per siliqua. The minimum phenotypic and genotypic coefficient of variability was observed for oil content followed by 1000-seed weight and plant height. Number of primary branches, number of secondary branches, seed yield per plant, days to 50% flowering, number of siliqua on main raceme, siliqua length and days to maturity also showed higher estimates of phenotypic and genotypic coefficient of variability.

Analysis of variance for the design used indicated highly significant differences for all the traits *viz.*, plant height, days to 50% flowering, days to maturity, number of siliqua on main raceme, number of primary branches, no. of secondary branches, 1000-seed weight, siliqua length, number of seeds per siliqua, oil content, seed yield per plant (Table 2). This indicates the presence of large amount of variability for all the characters. Generally these results are similar to those reported by Asghari *et al.*, (2011) and Sabaghnia *et al.*, (2010).

Heritability and genetic advance

The heritability value ranged from 30.6% (no. of secondary branches) to 98.8% (days to maturity). In general higher estimates of broad sense heritability were observed for all the traits. Moreover, the number of primary branches per plant, siliqua length, number of seeds per siliqua and seed yield per plant showed moderate broad base heritability while days to maturity exhibited the highest heritability. The genetic advance in percent of mean ranged from 5.27 % (oil content) to 42.54 % (days to 50% flowering) (Table 3). Plant height exhibited high heritability (79.6%) with high genetic advance in percentage of mean (21.48) for this trait might be taken into consideration while selecting a suitable line. High heritability was also calculated for this trait by Hasan *et al.*, 2014 and Yadava *et al.*, 2011. The high heritability (90.5%) along with considerable genetic advance in percentage of mean (22.90) provided opportunity for selecting high valued genotypes for 1000-seed weight. Singh *et al.*, (2002) reported the high heritability and genetic advance for 1000 seed weight. Seed yield exhibited moderate (63.4%) heritability with a high genetic advance in percentage of mean (31.52) indicating that phenotypic selection for seed yield per plant would be effective. Sharafi *et al.*, (2015) found similar result while Aytaç and Kinaci (2009) mentioned the high heritability and genetic advance for seed yield.

Correlation coefficient

Days to 50% flowering showed significant positive association with days to maturity and seed yield per plant at both genotypic and phenotypic level. These results suggested that if days to 50% flowering increased, then days to maturity and seed yield per plant also increased. Similar result was found by Ghosh and Gulati (2001). While days to maturity also showed significant positive correlation with plant seed yield per plant at both genotypic and phenotypic level. Plant height showed highly significant positive correlation with seed yield. Significant positive correlation between plant height and seed yield per plant was also found by Khayat *et al.*, (2012). Number of siliqua on main raceme showed significant positive correlation with seed yield per plant at both genotypic and phenotypic level. Thousand seed weight showed significant positive correlation with seed yield per plant at both genotypic and phenotypic level as reported by Tuncturk *et al.*, (2007). Seed yield per plant had highest significant positive correlation with plant height followed by days to maturity at both genotypic and phenotypic level suggesting, if the plant height and days to maturity increase then seed yield per plant also increases. Jeromel *et al.*, (2007) found complete positive correlation between plant height and yield (Table 4 and 5).

Table.1 Genotypes with their pedigree used for diversity analysis

S. No	Genotypes	Species	Pedigree	Source
1	CNH-11-7	*Brassica napus*	OCN8NA X China 1006BCR	PAU Ludhiana
2	HNS0901	*Brassica napus*	Selection from exotic selection	CCS HAU, Hisar
3	CNH-11-1	*Brassica napus*	Ag Outback NA X China 6 1006 NAR	PAU Ludhiana
4	CNH-11-13	*Brassica napus*	RT108NA X China 1006BCR	PAU Ludhiana
5	HNS1001	*Brassica napus*	HNS0004 X EC552585	CCS HAU, Hisar
6	GSL-1	*Brassica napus*	Selection from farmer'^s field	PAU Ludhiana
7	GSC-101	*Brassica napus*	Rivette X RR001	PAU Ludhiana
8	CNH-11-2	*Brassica napus*	ECN 3 NA X China 6- 1006 NAR	PAU Ludhiana
9	GSC-6	*Brassica napus*	-	PAU Ludhiana
10	NUDB2611QC	*Brassica napus*	-	Faizabad.
11	EC552608	*Brassica napus*	An exotic line of Gobhi sarson	CSKHPKV
12	RSPN-29	*Brassica napus*	DGS-1 X GSL 1	SKUAST-J
13	RSPN-25	*Brassica napus*	*B. napus* x *B. hirta*	SKUAST-J
14	AKGS-3	*Brassica napus*	HPN-1-36-16-9	CSKHPKV
15	DGS-1	*Brassica napus*	Selection from exotic collection	SKUAST-J
16	RSPN-28	*Brassica napus*	DGS-1 X RSPN 25	SKUAST-J
17	CNH-55	*Brassica napus*	BCN61 X China 6A.	PAU Ludhiana
18	CNH-13-1	*Brassica napus*	BCN3575NA X China 6-1006-2	PAU Ludhiana.
19	PusaTarak	*Brassica juncea*	SEJ-8 X Pusa Jagannath	IARI New Delhi
20	PTC-2009-3	*Brassica campestris*	Composite(IGT-1+TS-29+TS-36+TS-38+TS-46+TS-50+Bhawani)	GB PUA & T, Pantnagar.
21	RSPT-2	*Brassica campestris*	Mass selection from local germplasm	SKUAST-J
22	Varuna	*Brassica juncea*	Selection from Varanasi Local 786,02.021976	Kanpur

Table.2 Analysis of variance for different characters in *Brassica* genotypes

Characters	Mean squares Replication	Treatment	Error
	d.f. 2	21	42
Plant height (cm)	22.10	1551.12**	121.95
No of primary branches/plant	0.195	9.80**	2.64
No of secondary branches/plant	0.195	9.57**	4.12
No of siliqua on main raceme	10.65	929.78**	76.19
Siliqualength(cm)	1.205	2.41**	0.59
No. of seeds /siliqua	6.695	63.16**	14.67
1000-seed weight(g)	0.11*	0.51**	0.02
Oil content(%)	0.065	3.27**	0.03
Days to 50% flowering	12.41	937.52**	5.55
Days to maturity	128.225**	1203.37**	4.78
Seed yield per plant(g)	4.57	24.73**	3.99

*=Significant at 5 per cent**= Significant at 1 per cent

Table.3 Mean and range for different characters of *Brassica* genotypes

Characters	**Grand mean** $(\bar{X}) \pm S.E.$	**Range**
Plant height (cm)	186.71±9.02	105-226
No of primary branches	9.21±1.32	04-13
No of secondary branches	15.11±1.65	10-20
No of siliqua on main raceme	84.92±7.12	44-122
Siliqua length (cm)	5.55±0.63	3.0-8.0
No. of seeds per siliqua	20.61±3.13	10-29
1000-seedweight (g)	3.49±0.14	2.9-5.0
Oil content (%)	40.0±0.14	38-42
Days to 50% flowering	84.59±1.92	37-101
Days to maturity	151.00±1.78	90-16
Seed yield per plant (g)	13.67±1.6	3.48-20.8

Table.4 Coefficient of variability, heritability and genetic advance in per cent of mean for different characters in *Brassica*

	Coefficients of variability		Heritability (h^2_{bs})	Genetic Advance
	Pcv	GCV	in % age	in % of Mean
Plant height (cm)	13.10	11.69	79.6	21.48
No of primary branches	24.34	16.79	47.4	23.77
No of secondary branches	16.13	8.92	30.6	10.19
No of siliqua on main raceme	22.36	19.86	78.9	36.33
Siliqua length (cm)	19.72	14.06	50.8	20.73
No. of seeds per siliqua	26.95	19.51	52.4	29.12
1000-seed weight (g)	12.24	11.65	90.5	22.90
Oil content (%)	2.63	2.60	97.4	5.27
Days to 50% flowering	20.02	20.84	98.2	42.54
Days to maturity	13.32	13.24	98.8	27.10
Seed yield per plant (g)	24.14	19.22	63.4	31.52

Analysis of variance

Sources of variations	**d.f**	**Mean squares variances**	
		Observed	**Expected**
Replications	(r-1)	M_r	
Treatments	(t-1)	M_v	$\sigma^2 e_{ii} + r\sigma^2_{ii}$
Error	(r-1)(t-1)	M_e	$\sigma^2 e_{ii}$

Table.5 Genotypic and phenotypic correlations coefficients for different characters of *Brassica* genotypes

Characters		X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
X_1-Plant height(cm)	r_p	0.093	0.014*	0.283*	0.256*	0.277*	-0.195	-0.270*	0.712**	0.817**	0.294*
	r_g	-0.030	0.004*	0.291*	0.394*	0.438*	-0.236	-0.306	0.802*	0.916**	0.379*
X_2-No. of pri. branches			0.266*	-0.010	0.190*	0.057*	0.049*	-0.006	0.155*	0.160*	0.036*
			0.538**	-0.254	0.321*	0.055*	0.099*	-0.009	0.238*	0.238*	0.146
X_3-No. of Sec. branches				0.010*	0.161*	-0.073	-0.163	-0.063	0.018*	0.114*	0.020
				0.045*	-0.212	-0.578	-0.355	-0.136	0.033*	0.174*	0.172
X_4-No. of siliqua on m.r.					0.245*	0.112*	-0.362	0.012*	0.299*	0.289*	0.280*
					0.312*	0.034*	-0.415	-0.003	0.342*	0.337*	0.360*
X_5-Siliqua length(cm)						0.597**	-0.287	-0.129	0.532*	0.485*	-0.086
						0.873**	-0.424	-0.181	0.713**	0.684**	-0.236
X_6-No. of seeds per siliqua							0.035*	-0.190	0.570**	0.497*	0.012
							0.066*	-0.276	0.767**	0.711**	0.056
X_7-1000 seed weight (g)								0.144*	-0.213	-0.225	0.077*
								0.161*	-0.219	-0.249	0.099*
X_8- oil content (%)									-0.276	-0.408	0.093*
									-0.281	-0.421	0.112*
X_9-days to50%flowering										0.933**	0.153*
										0.949**	0.193*
X_{10}-days to maturity											0.148*
											0.206*

X_{11} = Seed yield per plant (g)
*=Significant at 5 per cent, **= Significant at 1 per cent

Table.6 Direct (diagonal) and indirect (off diagonal) effects of different characters on seed yield per plant at genotypic level

characters	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}
Plant height (cm)	1.219	0.000	-0.001	0.086	0.232	-0.432	-0.148	0.061	0.951	-1.590
No. of primary Branches/plant	-0.036	0.012	-0.091	-0.076	0.189	-0.054	0.062	0.002	0.282	0.412
No. of secondary Branches/plant	0.005	-0.007	0.169	0.013	-0.125	0.569	-0.222	0.027	0.039	-0.302
No. of siliqua on main raceme	0.355	0.003	-0.008	0.297	0.184	-0.033	-0.260	0.001	0.406	-0.585
Siliqua length (cm)	0.480	-0.004	0.036	0.093	0.590	-0.860	-0.265	0.036	0.845	-1.186
No. of seeds per siliqua	0.534	-0.001	0.098	0.010	0.515	0.984	0.041	0.055	0.910	-1.233
1000-seed weight (g)	-0.288	-0.001	0.060	-0.123	-0.250	-0.065	0.626	-0.03	-0.260	0.432
Oil content(%)	-0.373	0.000	0.023	-0.001	-0.107	-0.272	0.111	-0.199	-0.334	0.730
Days to 50% flowering	0.997	-0.003	-0.006	0.102	0.420	-0.755	-0.137	0.056	1.186	-1.647
Days to maturity	1.117	-0.003	-0.029	0.100	0.403	-0.700	-0.156	0.084	1.126	-1.735

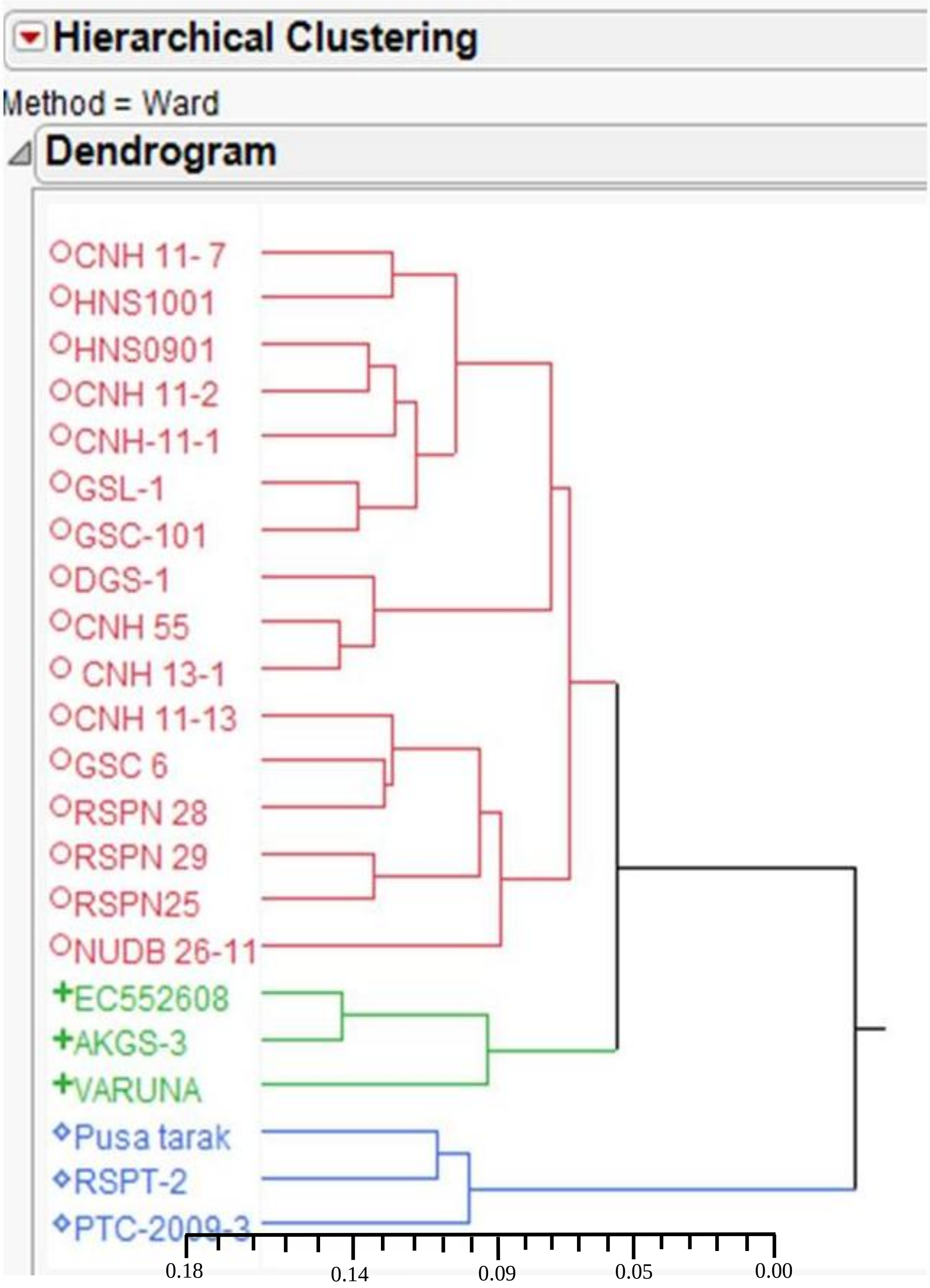
Residual = 0.329; Underline values denotes direct path effects.

Table.7 Direct (diagonal) and indirect (off diagonal) effects of different characters on seed yield per plant at phenotypic level

characters	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}
Plant height (cm)	**0.471**	**0.002**	**0.001**	**0.085**	**-0.042**	**-0.005**	**-0.038**	**-0.016**	**0.249**	**-0.414**
No. of primary branches/plant	**0.044**	**0.024**	**0.020**	**-0.003**	**-0.031**	**-0.001**	**0.009**	**0.000**	**0.054**	**- 0.081**
No. of secondary Branches/plant	**0.007**	**0.006**	**0.076**	**0.003**	**-0.026**	**0.001**	**-0.032**	**-0.004**	**0.006**	**0.058**
No. of siliqua on main raceme	**0.133**	**0.000**	**0.001**	**0.299**	**-0.040**	**-0.002**	**0.070**	**0.001**	**0.105**	**-0.146**
Siliqua length (cm)	**0.121**	**0.005**	**0.012**	**0.073**	**-0.163**	**-0.010**	**-0.056**	**-0.008**	**0.186**	**- 0.246**
No. of seeds per siliqua	**0.130**	**0.001**	**-0.006**	**0.033**	**-0.098**	**0.017**	**0.007**	**-0.011**	**0.200**	**-0.252**
1000-seed weight (g)	**-0.092**	**0.001**	**-0.012**	**-0.108**	**0.047**	**-0.001**	**0.195**	**0.008**	**-0.075**	**0.144**
Oil content (%)	**-0.127**	**0.000**	**-0.005**	**0.004**	**0.021**	**0.003**	**0.028**	**0.059**	**-0.097**	**0.207**
Days to 50% flowering	**0.335**	**0.004**	**0.001**	**0.090**	**-0.087**	**-0.010**	**-0.042**	**-0.016**	**0.350**	**-0.473**
Days to maturity	**0.385**	**0.004**	**0.009**	**0.086**	**-0.079**	**-0.008**	**-0.044**	**-0.024**	**0.327**	**-0.507**

Residual effect = 0.585; Underline values denotes direct path effects.

Fig.1 Dendrogram constructed for 22 oilseed rape cultivars based on morphological traits



Path coefficient

Days to 50% flowering had positive direct effect on seed yield per plant (1.186) at genotypic level. The highest indirect positive effect was found via plant height (1.17) followed by siliqua length (0.420). Days to maturity had negative direct effect on seed yield per plant (-1.735) and it also had positive correlation with seed yield per plant. Days to maturity had positive direct effect on seed yield through plant height (1.117), number of siliqua on main raceme (0.100), siliqua length (0.403), and oil content (0.084) and days to 50% flowering (1.126).

Plant height had direct positive effect (1.219) on seed yield per plant. These results indicated if plant height increased then seed yield also increased mostly through the direct positive effect of plant height and positive indirect effect of other characters. Aytac *et al.*, (2008) reported plant height showed a considerable direct positive effect on seed yield per plant. Number of primary branches per plant had positive direct effect on seed yield per plant and also positive highly significant correlation with seed yield per plant at genotypic level. Mahak *et al.*, (2003) reported that number of primary branches per plant had direct positive effect on seed yield. So, selection for this trait will be judicious and more effective in future breeding program.

Number of secondary branches per plant showed negative direct effect (-0.169) on seed yield per plant. The genotypic correlation with seed yield was positive mainly due to negative direct effect of number of secondary branches per plant plus positive indirect effect of other characters. Siliqua length had positive direct effect (0.590) on seed yield per plant (Tables 6 and 7). The genotypic correlation with seed yield was positive (0.236). Hence, selection should be practiced for this trait which had longer siliqua in order to improve seed yield. Number of seeds per siliqua had negative direct effect on seed yield per plant. This indicated that selection for greater number of seeds per siliqua would give better response in the improvement of seed yield per plant. Afrin *et al.*, 2011 found similar results for these traits.

Dendogram analysis

A dendogram was constructed by hierarchical clustering using ward’s method. Dendogram showed that the genotypes were divided into 3-groups. First group contains three genotypes viz. Pusa tarak, RSPT-2, PTC-2009-3; second group contained three genotypes viz. EC552608, AKGS-3 and Varuna; while the third group contained 16 genotypes.

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