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Multivariate analysis of some important quantitative traits in rapeseed (*Brassica napus* L.) advanced lines

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Abstract

Development of new rapeseed (*Brassica napus* L.) cultivars requires efficient tools to monitor traits relationship in a breeding program. Twenty four rapeseed genotypes including 2 cultivars and 22 advanced lines, were evaluated based on randomized complete block design with three replications. Significant genotypes effects were exhibited for phenological traits, plant height, yield components, and seed yield, indicating significant genetic differences among the genotypes. High broad sense heritability were estimated for phenological traits, pods on main axis and, seed yield, signifying selection gain for improving these traits. Duration of flowering and pods on main axis had high value of genetic coefficient of variation. The results of factor analysis exhibited four factors including sink factor (pod per plant, pods length and seed yield), fixed capital factor (phenological traits), secondary fixed capital factor (duration of flowering), and metric factor (plant height). The results of stepwise regression analysis revealed that plant height and pods per plant significantly had more decreasing and increasing effects respectivelyon seed yield. On the basis of cluster analysis, the genotypes were classified in four groups, and the group with high seed yield had high mean value of pods per plant.

Key words:coefficient of variation, cluster analysis, factor analysis, and stepwise regression.

Introduction

Rapeseed is grown as a high quality source of vegetable oil for the food industry and supplies protein to the animal feed market (Khachatourians *et. al.*, 2001; Mahasi and Kamundia, 2007). It is also becoming a popular oilseed crop in Iran, including north provinces, due to its high oil and protein contents. Improvement of seed yield in rapeseed (*Brassica na*pus L.) has been the primary objective of rapeseed breeders for many years. To increase the yield, study of direct and indirect effects of yield components provides the basis for its successful breeding program, and hence, the problem of yield increase can be more effectively tackled on the basis of performance of yield components, and selection for closely related characters (Aytac *et al.*, 2008; Marjanovic-Jeromela *et al.*, 2009). The multivariate analysis, particularly factor and cluster analyses, are utilized for evaluation of germplasm for various traits in a large number of accessions. Cluster analysis assigns genotypes into qualitative homogenous groups based on response similarities, and also assists to classify genotypes. The method among group means produces a dendrogram showing successive fusion of individuals. Greater heterotic effect is generated when clusters are divergent. Genetic diversity among the Brassica genotypes was assessed by Choudhary and Joshi (2001) using cluster analysis. The morphological characters viz., days to flowering, plant height, secondary branches per plant, and 1000-seed weight contributed maximum towards genetic divergence (Leilah and Al-Khatee 2005; Aytac and Kýnaci 2009). Determination of correlation coefficients is an important statistical procedure to evaluate breeding programs for high yield, as well as to examine direct and indirect contributions to yield variables (Khan *et al.*, 2006; Ivanovska *et al.*, 2007; Basalma, 2008; Hashemi *et al.*, 2010; Semahegn Belete, 2011). Zang and Zhou (2006) reported that pods per plant, seeds per plant, and 1000-seed weight traits were positively correlated with seed yield. On the other hand, Length of pod was negatively correlated with seed yield. Jeromela et al. (2007) studied 30 rapeseed varieties and demonstrated that pods per plant have the highest correlation with seed yield. Khan *et al.* (2008) also reported the positive significance correlation between seed yield and plant height, pods per plant, seeds per pod, and pod length. Sheikh *et al.* (1999) found high heritability estimates coupled with high genetic advanced for seed yield per plant, pods per plant and seed weight in rapeseed (Brassica campestris) genotypes. They also reported positive correlation of all the yield components with seed yield. Genetic variability is prerequisite for improving any crop plant.

The main applications of factor analytic techniques are to reduce the number of variables, and to detect structure in the relationship between variables, that is to classify variable (Sharma, 1996). In plant breeding factor analysis is mainly applied as structure detection method, and sometimes it can be used as index selection for improving more than one trait. Factor analysis was used to determine structural factors related to growth trait and yield components, and also it was used for detecting factors relating to environmental stress including drought resistance in *Brassica napus* (Naderi and Emam, 2010).

The objectives of the present study were to estimate the genetic coefficient of variation, broad sense heritability, relationship among yield associated traits for improving seed yield in spring type of rapeseed advanced lines and cultivars, and also classify the genotypes via factor and cluster analyses.

Materials and Methods

The material under study consisted of 24 rapeseed (*B. napus* L.) genotypes which were selected based on different agronomic characters. The genotypes were evaluated based on randomized complete block design with three replications at Baykola Agriculture Research Station, located in Neka, Iran (53Ú, 13² E longitude and 36Ú 43² N latitude, 15 m above sea level) during 2011-12. The plots consisted of four rows 5 m long and 30 cm apart. The distance between plants on each row was 5 cm resulting in approximately 400 plants per plot, which were sufficient for statistical analysis. Crop management factors like land preparation, crop rotation, fertilizer, and weed control were followed as recommended for local area. All the plant protection measures were adopted to make the crop free from insects. Phenological traits including days to flowering, days to end of flowering, duration of flowering, and days to maturity were determined based on phenological stages of the genotypes in each plot. Pods per main axis, pods length and pods per plant were recorded based on 10 randomly plants of each plot. Seed yield (adjusted to kg/ha) was recorded based on two middle rows of each plot.

Variance components were estimated from the mean squares in the analysis of variance (Singh *et al.*,1993). The component of variance including error variance (VE), genotypic variance (VG) and phenotypic variance (VP) were estimated according to the following formula:

$$VE = MSE$$

$$VG = (MSG - MSE)/r$$

$$VP = VG + VE$$

Broad sense heritability (h2) was estimated according to Singh *et al.*, (1993) as: h^2=VG/VE. The coefficient of variation was estimated as CV=$(\sqrt{VG})/\mu$ in which μ is the mean of genotypes for each trait.

The correlation coefficients between the traits were estimated and then factor analysis on the base of major factors analysis and varimax rotations was done on the data. Principal components method analysis was used to extract factorial load of matrix and also to estimate the number of factors (Sharma. 1996). Therefore, the factors which had a root bigger than one were selected and were used to form factorial coefficients matrix. By means of varimax rotation, rotation was done on the major factorial loads matrix and the matrix of rotated factorial loads was obtained (Sharma. 1996; Rameeh, 2010). The average-linkage-between-groups method of cluster analysis, often aptly called UPGMA (un weighted pair-group method using arithmetic averages) was used, which defines the distance between two clusters as the average of the distances between all pairs of cases in which one member of the pair is from each of the clusters.. All the analyses were performed using MS-Excel and SAS software version 9 (SAS INSTITUTE INC, 2004).

Results and Discussions

Analysis of variance

Significant mean square of genotypes were determined for the traits including days to flowering, days to end of flowering, duration of flowering, days to maturity, plant height, pods per main axis, pods per plant, pods length, 1000-seed weight and seed yield indicating significant genetic variation for these traits (Table 1). Generally these results are similar to those reported by Aytac and Kinaci (2009), and Sabaghnia, et. al., (2010). Broad sense heritability estimates ranged from 0.21 to 0.94 for pods per plant and days to flowering, respectively. High broad sense heritability estimates for the phenological traits, and seed yield, indicate selection gain for improving these traits. Aytaç et al. (2008) also reported high broad sense heritability estimates for yield components. Genetic coefficient of variation, an indicator of the genetic diversity of the genotypes, was varied from 0.05 to 43.66 related to pods per plant and duration of flowering, respectively (Table 2). The high value of genetic variations of the genotypes were detected for pods length, pods on main axis, and duration of flowering.

Correlation among the traits and means of the genotypes

Mean value of days to flowering ranged from 151 to 178 days in G1 and G6, respectively (Table 2). Significant positive correlation was detected between days to flowering and days to maturity (Table 3), which therefore, suggest that selection for this trait should be done for early maturity genotypes. The genotypes including G2, G9, G13, G14, G19, G20, G22 and G24, with low means values are prefered for selection related to days to end of flowering; genotypes G2, G9, G13, G22 and G24 with low mean values for days to maturity are

Table 1- Randomize complete block (RCBD) analysis of variance for the studied traits.

S.O.V	df	MS									
		Days to flowering	Days to end of flowering	Duration of flowering	Days to maturity	Plant height	Pods per main axis	Pods per plant	Pods length	1000 seed weight	Seed yield
Replication	2	0.10	3.4*	2.6	1.8	935.5**	20.4	4560.6**	0.45	2.31**	2474595**
Treatments	23	107.7**	69.1**	79.1**	57.4**	420.6**	263.7**	584.9*	1.13**	0.11**	460219**
Error	46	2.40	0.75	3.2	3.5	140.1	68.13	324.43	0.39	0.05	70560
Broad-sense heritability		0.94	0.97	0.89	0.84	0.40	0.49	0.21	0.39	0.29	0.65

suitable genotypes for improving this trait. Due to low mean value of genetic coefficient of variation for days to end of flowering, selection gain for improving this trait will be low. In rapeseed, since flowering and seed formation occurred about the same time, tgenotypes showing high mean values of duration of flowering will be preferable. High mean value of duration of flowering suggests that the plant gets enough time and opportunity for seed formation, and therefore the correlation between duration of flowering and 1000-seed weight was positive. The genotypes G1, G4 and G7 with high mean value of duration of flowering were considered most suitable genotypes for improving this trait. For reducing some of pests damages and also having enough opportunity for second crop cultivation after rapeseed, early maturity is ideotype trait for breeding *Brassica napus* L. and other related brassica specious. Plant height ranged from 111 to 156 cm in G5 and G23, respectively. For ideotype breeding of rapeseed, low mean value of plant height is favored, and thus G5, G14 and G20 were merit genotypes for this trait. Since pods per main axis was found significantly correlated with seed yield, the genotypes G1, G3, G4, G11 and G19 were suitable for improving this trait. Since pods per plant is an important yield component, genotypes G1, G7, G19 and G24 with high mean values are prefered; genotypes G1, G7, G19 and G24 which have high mean values of pods length and 1000-

Table 2: Mean comparison of the rapeseed genotypes for phenologlical traits, plant height, yield components and seed yield.

Traits/ Genotypes	**Days to flowering**	**Days to end of flowering**	**Duration of flowering**	**Days to maturity**	**Plant height (cm)**	**Pods per main axis**	**Pods per plant**	**Pods length**	**1000 seed weight (g)**	**Seed yield (kg/ha)**
1-Fornex/ 401(G1)	151	206	55	238	147	68	155	6.97	3.88	3056
2-Zarfam/401(G2)	159	197	39	224	141	45	116	5.03	3.39	2110
3-Zarfam/308(G3)	162	199	37	228	143	70	124	5.17	3.87	2128
4-Fornex /401(G4)	164	206	42	237	126	56	142	6.08	3.93	2928
5-Fornex/308(G5)	166	204	38	233	111	40	134	6.03	3.64	2956
6-Folesta/308(G6)	178	211	33	239	142	51	138	5.04	3.37	2805
7-Modena/308(G7)	166	207	41	233	143	52	153	6.10	3.81	2978
8-Opera/308(G8)	176	208	32	238	129	39	121	5.20	3.60	1906
9-Slm046/401(G9)	166	198	32	227	143	59	135	5.29	3.57	2478
10-Zarfam/401(G10)	166	200	34	231	154	41	133	4.84	3.68	2217
11-Zarfam/401(G11)	168	204	36	232	149	59	148	5.53	3.75	2733
12-Slm046/401(G12)	168	206	38	231	138	38	138	5.45	3.57	2333
13-Okapi/308(G13)	169	198	29	226	122	50	125	4.88	3.78	2278
14-Okapi/308(G14)	169	198	29	230	115	50	115	4.59	3.70	2370
15-Okapi/308(G15)	170	206	36	232	127	37	114	5.27	3.53	1767
16-Slm046/308(G16)	168	204	36	232	124	47	124	5.92	3.84	2206
17-Folesta/401(G17)	177	211	33	240	139	44	116	4.71	3.44	2333
18-Sarigol(G18)	172	208	36	235	134	42	125	4.92	3.74	2247
19-Hsari(G19)	165	198	33	230	133	59	155	6.00	3.93	2924
20-Hyola401(G20)	160	196	36	227	121	40	132	5.53	3.62	2839
21-RGS003(G21)	161	199	38	231	136	49	119	5.24	4.07	2586
22-Zodras22(G22)	165	198	34	228	147	63	152	6.32	3.65	2956
23-Zodras13(G23)	170	208	38	236	156	45	127	4.81	3.66	2117
24-Zafar(G24)	165	198	33	229	134	52	155	6.25	4.10	3015
Genetic coefficient of variation	5.94	2.90	43.66	2.74	6.35	22.22	0.05	9.24	4.19	1.16
LSD$_{(\text{á}=0.05)}$	2.53	1.41	2.92	3.06	19.33	13.48	29.41	1.02	0.37	433.77
LSD$_{(\text{á}=0.01)}$	3.36	1.88	3.89	4.06	25.71	17.93	39.12	1.36	0.49	576.92

Table 3. Correlation among the traits in rapeseed.

Traits	1-Days to flowering	2-Days to end of flowering	3-Duration of flowering	4-Days to maturity	5-Plant height (cm)	6-Pods per main axis	7-Pods per plant	8- Pods length	9-1000 (g) seed weight	10-Seed yield (kg/ha)
1-Days to flowering	1									
2-Days to end of flowering	0.57**	1								
3-Duration of flowering	-0.64**	0.27	1							
4-Days to maturity	0.45*	0.89**	0.31	1						
5-Plant height	-0.06	0.18	0.24	0.12	1					
6-Pods per main axis	-0.44*	-0.28	0.26	-0.13	0.34	1				
7-Pods per plant	-0.21	-0.05	0.32	0.04	0.31	0.52**	1			
8- Pods length	-0.56**	-0.06	0.59**	0.04	-0.01	0.45*	0.78**	1		
9-1000-seed weight	-0.44*	-0.31	0.22	-0.11	-0.09	0.44*	0.40	0.47*	1	
10-Seed yield	-0.38	-0.14	0.31	0.05	-0.02	0.46*	0.81**	0.72**	0.39	1

*, ** Significant at p=0.05 and 0.01, respectively.

seed weight are also suitable for breeding. Zhang and Zhou (2006) reported that pods per plant, seeds per plant and 1000-seed weight traits were positively correlated with seed yield. Jeromela et al. (2007) studied 30 rapeseed varieties and demonstrated that pods per plant have the highest correlation with seed yield. Khan et al. (2006) also reported the positive significant correlation between seed yield and plant height, pods per plant, seeds per pod and pod length. The genotypes G1, G4, G7, G19 and G24 with high mean values of seed yield (4089, 4117, 4094 and 4289 kg/ha, respectively) also had high mean values for some other yield components.

Multivariate analyses

The results of stepwise regression analysis indicated plant height and pods per plant, respectively had decreasing and increasing effects on seed yield (Table 4).

The results of factor analysis based on minimum eigenvalue revealed four factors for 10 studied traits (Table 5). The eigenvalues for factor one to four were 3.99, 3.32, 1.17 and 1.06, respectively. The cumulative variation for these factors was 0.86 and also it's portions for factor one to four were 0.39, 0.23, 0.12 and 0.11, respectively. Factor one was detected as " Sink Factor" in which, pod per plant, pods length and seed yield had high coefficients for factor loading. The second, third and fourth factors were named " Fixed Capital Factor" for phenological traits, "Secondary Fixed Capital Factor" for duration of flowering and "Metric Factor" for plant height, respectively. In earlier studies (Naderi and Emam, 2010; Rameeh, 2010) factor analyses were used to determine structural factors related to growth trait and yield components, and also for detecting factors relating to environmental stress including drought resistance in *B. napus* L.

The results of cluster analysis for 10 studied traits in 24 genotypes are presented in Figure 1 and Table 6. All the genotypes were classified in four groups with different mean values of the traits. The high seed yield genotypes with high mean value of pods per plant were classified in group1(C1). Group 2

(C2), group3 (C3) and group 4(C4) had 2234, 2532 and 1836 kg ha^{-1} of seed yield, respectively. Genetic diversity among the Brassica genotypes was assessed by Choudhary and Joshi (2001) using cluster analysis.

In general the phenological traits, pods on main axis and seed yield were more heritable than the other traits. Due to significant positive correlation of yield components with seed yield these traits can be used as indirect selection criteria for improving seed yield. The low value of genetic coefficient of variation for days to maturity suggests that for improving this trait, the correlated traits including days to flowering and days to end of flowering can be used. Cluster analysis can be used as suitable method for classifying the high yield genotypes.

Table 4. The results of stepwise regression analysis of 10 studied traits.

Step	Variable entered	Partial R-square	Model R-square	F-test
1	X_5:plant height	0.66	0.66	43.77**
2	X_7:pods per plant	0.08	0.74	6.46*

Final regression model: Y(seed yield)= $449.87-9X_5+25.41X_7$

Table 5: Factor analysis for 10 studied traits in rapeseed genotypes.

	Factor loadings			
Traits	**1**	**2**	**3**	**4**
Days to flowering	-0.30	0.54	-0.78	-0.07
Days to end of flowering	-0.13	**0.97**	0.01	0.07
Duration of flowering	0.22	0.29	**0.91**	0.15
Days to maturity	0.06	**0.95**	0.05	0.01
Plant height	-0.01	0.13	0.08	**0.95**
Pods per main axis	0.57	-0.28	0.15	0.52
Pods per plant	**0.90**	0.05	0.06	0.29
Pods length	**0.80**	0.04	0.47	-0.06
1000-seed weight	0.58	-0.28	0.24	-0.13
Seed yield	**0.91**	0.01	0.09	-0.02
Eigen value	3.99	2.32	1.17	1.06
Portion	0.39	0.23	0.12	0.11
Cumulative	0.40	0.63	0.75	0.86

Table 6:The means of clustering groups for 10 studied traits.

Traits Clustering groups	Days to flowering	Days to end of flowering	Duration of flowering	Days to maturity	Plant height (cm)	Pods per main axis	Pods per plant	Pods length (cm)	1000 seed weight (g)	Seed yield (kgha^{-1})
C1	163	202	39	232	134	54	147	6.09	3.81	2931
C2	168	203	35	231	137	47	124	5.03	3.67	2234
C3	164	199	35	229	139	54	127	5.27	3.82	2532
C4	173	207	34	235	128	38	118	5.23	3.57	1836

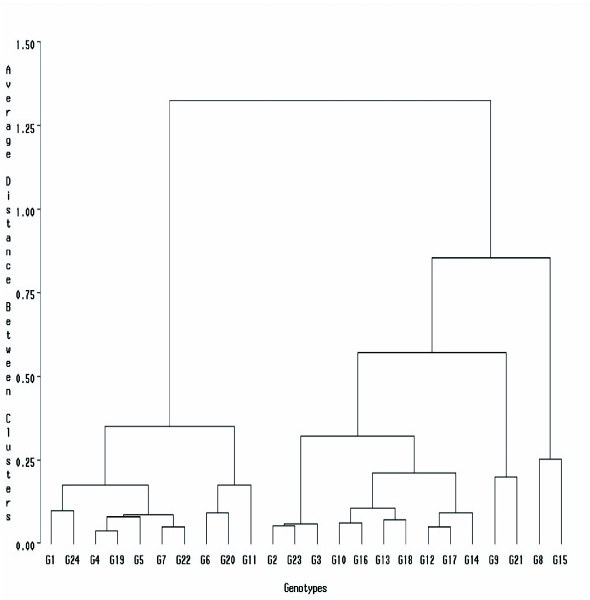

Figure 1-Clustering dendrogram of 24 rapeseed genotypes (the genotypes code as in Table 2) based on 10 studied traits.

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