

GENETIC DIVERSITY ANALYSIS FOR AGRO-MORPHOLOGICAL AND SEED QUALITY TRAITS IN RAPESEED (*BRASSICA CAMPESTRIS* L.)

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Abstract

One hundred fourteen accessions of rapeseed (*Brassica campestris* L.) were evaluated at National Agricultural Research Centre, Islamabad, Pakistan using cluster and principal component analyses during 2005 and 2006. Cluster analysis based on fifteen agro-morphological and six seed quality traits, divided 114 accessions into six and five clusters during 2005 and 2006, respectively. The first seven and five PCs with eigenvalues > 1 contributed 74.09% and 66.08% of the variability amongst accessions during 2005 and 2006, respectively. Nine important characters contributed positively to first two PCs during both the years 2005 and 2006.

Introduction

In Pakistan mainly two types of oilseed crops i.e., traditional (rapeseed-mustard, groundnut and sesame) and non-traditional (sunflower, safflower and soybean) are grown. Rapeseed-mustard is the major contributor among traditional oilseed crops (Ali & Mirza, 2005). Rapeseed-mustard is being grown on marginal lands scattered in whole Pakistan on considerable area of 228 thousand hectares (Anon., 2008).

The multivariate analysis particularly the principal component and cluster analyses are utilized for evaluation of germplasm when studying various traits and a large number of accessions. Genetic diversity among the *Brassica* genotypes was assessed by Choudhary & 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. Principal component and cluster analyses disclosed complex relationships among 36 Ethiopian mustard accessions evaluated by Alemayehu & Becker (2002). Yield components and length of growing period contributed most for divergence and clustering pattern. The genetic diversity among the genotypes of *Brassica campestris* var. sarson (gobhi sarson) was attributed to the number of secondary branches per plant, number of days to 50% flowering, 1000-seed weight, oil percentage, number of siliquae per plant and plant height (Jeena & Sheikh, 2003). The genetic diversity and relationships among 63 rapeseed accessions were evaluated by Wu *et al.*, (2007) for nine agronomical important characters using cluster analysis which clearly separated the European accessions from the Chinese ones. However, the Chinese accessions with erucic acid free and/or low glucosinolates could not be separated from those Chinese accessions with both high erucic acid and glucosinolates. The present studies were carried out to investigate the extent of genetic diversity in rapeseed (*Brassica campestris* L.) germplasm based on agro-morphological and seed quality traits using multivariate analysis.

Materials and Methods

One hundred fourteen accessions of rapeseed (*Brassica campestris* L.) were evaluated for fifteen agro-morphological and six seed quality traits under field conditions

using augmented design, including a check variety BSA during the years 2005 and 2006 at National Agricultural Research Center, Islamabad, Pakistan. Each accession was planted in 3 rows of 4 meter length. Row to row and plant to plant spacing was kept at 45 and 10 cm, respectively. Check variety BSA was included and repeated after every 10 accessions. For seed bed preparation pre-sowing irrigation was applied to plant experiment under optimum moisture conditions. Afterwards the experiments were grown under rain fed conditions during both seasons. Fertilizer @ 90 kg N and 60 kg P₂ O₅ was applied at the time of land preparation. Planting of the experiments was done with hand drill at 3-4 cm depth. Thinning was done to maintain optimum plant population. Weeds were controlled by hand weeding. The data were recorded on ten randomly sampled plants from each accession for days to flower initiation, days to flower completion, days to maturity, plant height (cm), number of primary branches per plant, number of secondary branches per plant, length of main inflorescence (cm), siliqua length (cm), siliqua width (cm), siliqua length/width ratio, number of Siliqua per main inflorescence, number of siliqua per plant, number of seeds per siliqua, 1000-seed weight (g), seed yield per plant (g), oil contents (%), protein contents (%), oleic acid contents (%), linolenic acid contents (%), erucic acid contents (%) and glucosinolates contents (μMg^{-1}). Seed quality traits were determined by Near Infrared-Reflectance spectroscopy (NIRS) at Nuclear Institute for Food and agriculture (NIFA), Tarnab, Peshawar. Cluster and principal component analyses were carried out based on fifteen agro-morphological and six seed quality traits for the years 2005 and 2006 separately using “Statistica” Version 6.0 and “SPSS” for Windows Xp, respectively.

Results and Discussion

Cluster analysis based on fifteen agro-morphological and six seed quality traits, during 2005, divided 114 accessions of *Brassica campestris* L., into six clusters. Mean values and standard deviations for each of 6 clusters are presented in Table 1. Cluster I consisted of 26 accessions and these were late in flowering, late in maturity (170.65 days), tall (151.13 cm), medium high number of siliqua per plant (258.30), high seed yield per plant (23.81 g) and high oil contents (46.21%). Cluster II comprised of 19 accessions and these accessions were late in flowering, late in maturity (169.32 days), very tall (166.45), very high number of siliqua per plant (426.81), high seed yield per plant (29.33 g) and comparatively low oil contents (44.46%). There were six accessions in cluster III and these accessions were characterized by early flowering but late maturing (170.17 days), very tall (171.33 cm), high number of siliqua per plant (300.00), high 1000 seed weight (4.01 g), low seed yield per plant (11.40 g), very high oil contents (48.88%), low protein contents (26.00%) and very high glucosinolates contents ($102.93 \mu\text{Mg}^{-1}$). Cluster IV consisted of four accessions and these accessions were late in flowering, late in maturity (172.75 days), tall (150.46 cm), medium high number of siliqua per plant (283.13), high 1000 seed weight (4.19 g), low seed yield per plant (13.33 g), very high oil contents (49.40%), low protein contents (22.65%) and high glucosinolate contents ($85.35 \mu\text{Mg}^{-1}$). There were 37 accessions in cluster V and these accessions were early in flowering, medium late in maturity (159.59 days), less tall (128.66 cm), low number of siliqua per plant (249.01), low 1000 seed weight (2.85 g), low seed yield per plant (13.05 g), very high oil contents (48.29%), low protein contents (24.68%) and high glucosinolates contents ($98.08 \mu\text{Mg}^{-1}$). Cluster VI had 22 accessions and these accessions were characterized by early in flowering, medium late in maturity (160.64 days), medium tall (133.99 cm), medium high number of siliqua per plant (282.17), low 1000 seed weight (2.89 g), high seed yield per plant (19.44 g), high oil contents (46.76%), medium high protein contents (25.45%) and high glucosinolates contents ($106.54 \mu\text{Mg}^{-1}$).

Table 1. Means values and standard deviations (SD) of 6 clusters based on 15 agro-morphological and 6 seed quality traits of 114 accessions of *Brassica campestris* L., during 2005.

Variables	Cluster I (26 accessions)		Cluster II (19 accessions)		Cluster III (6 accessions)		Cluster IV (4 accessions)		Cluster V (37 accessions)		Cluster VI (22 accessions)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Days to flower initiation	85.65	12.08	97.00	13.58	63.83	8.11	82.75	9.46	71.35	16.27	63.36	11.16
Days to flower completion	146.77	5.91	146.89	4.88	121.67	1.97	152.00	6.58	126.32	11.41	123.09	7.73
Days to maturity	170.65	4.00	169.32	4.47	170.17	4.75	172.75	5.38	159.59	4.43	160.64	6.84
Plant height (cm)	151.13	29.89	166.45	15.88	171.33	18.34	150.46	19.57	128.66	21.94	133.99	25.43
Number of pri. Br. per plant	4.90	1.31	5.87	1.88	3.00	1.30	7.00	0.98	5.19	1.45	4.93	1.10
Number of sec. br. per plant	12.94	3.04	15.55	4.64	7.50	2.61	16.06	3.60	13.65	2.42	13.00	2.71
Length of main inflorescence (cm)	46.43	8.14	52.60	10.74	58.83	7.23	43.32	7.35	45.56	7.60	50.20	7.47
Silique length (cm)	4.60	0.66	3.94	1.13	4.99	1.09	4.98	0.82	5.21	1.00	3.66	0.74
Silique width (cm)	0.31	0.05	0.31	0.05	0.41	0.08	0.28	0.04	0.32	0.04	0.34	0.04
Silique len/width ratio	15.43	3.48	12.97	4.44	12.51	3.34	18.17	3.16	16.59	3.88	10.94	2.20
No. of silique per main inflo	32.20	8.51	44.50	7.39	47.17	5.99	39.25	8.73	30.00	8.70	34.84	9.99
Number of silique per plant	258.30	69.65	426.81	140.26	300.00	73.48	283.13	76.63	249.01	86.05	282.17	102.94
Number of seeds per silique	18.63	3.64	17.66	3.25	21.25	3.34	16.00	0.20	21.42	4.52	16.89	1.94
1000seed wt. (g)	3.12	0.32	2.84	0.46	4.01	0.56	4.19	1.30	2.85	0.33	2.89	0.35
Seed yield/ plant (g)	23.81	7.24	29.33	6.03	11.40	6.82	13.33	13.94	13.05	8.73	19.44	9.35
Oil content %	46.21	1.21	44.46	1.17	48.88	1.64	49.40	1.45	48.29	1.34	46.76	2.08
Protein content %	25.32	1.19	26.69	0.98	24.00	1.63	22.65	1.29	24.68	1.18	25.45	1.94
Glucosinolates (μMg^{-1})	124.02	12.66	127.73	11.41	102.93	4.30	85.35	11.93	98.08	7.59	106.54	18.23
Oleic acid %	26.44	1.98	26.87	2.44	34.10	3.93	35.75	3.37	29.55	1.86	28.83	2.62
Linolenic acid %	9.75	0.89	9.51	0.98	7.82	0.63	7.93	0.29	8.27	0.83	8.67	0.89
Erucic acid %	52.58	1.80	53.33	1.27	52.92	0.85	42.48	5.17	53.07	1.18	52.95	1.64

Cluster analysis based on fifteen agro-morphological and six seed quality traits, during 2006, divided 114 accessions of *Brassica campestris* L., into five clusters. Mean values and standard deviations for each of five clusters are presented in Table 2. Cluster I consisted of 19 accessions and these were late in flowering, medium late in maturity (168.95 days), medium tall (130.62 cm), less number of siliqua per plant (191.36), lower seed yield per plant (8.76 g), high oil contents (46.17%), high protein contents (25.68%) and very high glucosinolates contents (128.01 μMg^{-1}). Cluster II comprised of 30 accessions and these accessions were late in flowering, medium late in maturity (166.83 days), medium tall (133.47 cm), less number of siliqua per plant (206.57), low seed yield per plant (14.08 g), high oil contents (45.97%) higher protein contents (27.47%) and very high glucosinolates contents (123.01 μMg^{-1}). There were seven accessions in cluster III and these accessions were characterized by medium late in flowering, medium late in maturity (169.71 days), medium tall (135.34 cm), less number of siliqua per plant (232.57), lower seed yield per plant (8.47 g), very high oil contents (49.13%), low protein contents (24.00%) and very high glucosinolates contents (103.14 μMg^{-1}). Cluster IV consisted of seven accessions and these accessions were late in flowering, medium late in maturity (166.86 days), less tall (127.11 cm), lowest number of siliqua per plant (131.03), lowest seed yield per plant (5.95 g), highest oil contents (50.13%), lowest protein contents (22.56%) and high glucosinolates contents (93.16 μMg^{-1}). Cluster V comprised of 51 accessions characterized as medium late in flowering, medium late in maturity (160.51), short stature (107.00 cm), less number of siliqua per plant (205.12), low 1000 seed weight (2.84 g), low seed yield per plant (10.44 g), very high oil contents (48.71%), medium high protein contents (25.11%) and high glucosinolates contents (98.38 μMg^{-1}).

The results of cluster analysis for the years 2005 and 2006 suggested that there is enough variation among the accessions for different agro-morphological and seed quality traits. Accessions with greater similarity for agro-morphological and seed quality traits were placed in the same cluster. Results of present studies are in agreement with those of Elizabeth *et al.*, (2001), Choudhry & Joshi (2001), Balkaya *et al.*, (2005) and Wu *et al.*, (2007).

Principal component analysis based on fifteen agro-morphological and six seed quality traits during 2005 revealed that seven of the 21 principal components with an eigenvalue higher than one accounted for 74.09% of the total variation among 114 accessions of *Brassica campestris* L. Coefficients defining seven principal components of the data are given in Table 3. The PC1 accounted for 23.55% of the total variation. The variation in PC1 was mainly attributed to days to flower initiation (0.670), days to flower completion (0.686), days to maturity (0.530), plant height (0.519), number of primary branches per plant (0.234), number of secondary branches per plant (0.215), length of main inflorescence (0.154), number of siliqua per main inflorescence (0.395), number of siliqua per plant (0.420), seed yield per plant (0.589), protein contents (0.664), glucosinolates contents (0.820), linolenic acid contents (0.600) and erucic acid contents (0.146). The PC2 contributed 11.77% of the total divergence and depicted the pattern of variation mainly in days to flower initiation (0.238), days to flower completion (0.466), days to maturity (0.333), plant height (0.117), number of primary branches per plant (0.435), number of secondary branches per plant (0.367), siliqua length (0.534), siliqua length/width ratio (0.757), number of seeds per siliqua (0.225), 1000 seed weight (0.253), oil contents (0.164) and oleic acid contents (0.622). The PC3 constituted 10.10% of the total variation and the variation was mainly attributed to days to maturity (0.203), plant height (0.299), length of main inflorescence (0.474), siliqua width (0.269), number of

Table 2. Means values and standard deviations (SD) of 5 clusters based on 15 agro-morphological and 6 seed quality traits of 114 accessions of *Brassica campestris* L., during 2006.

Variables	Cluster I (19 accessions)		Cluster II (30 accessions)		Cluster III (7 accessions)		Cluster IV (7 accessions)		Cluster V (51 accessions)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Days to flower initiation	86.37	14.98	84.10	16.33	64.57	7.70	77.00	11.08	72.10	16.87
Days to flower completion	145.32	8.56	141.20	10.99	124.71	2.36	146.71	9.96	127.33	10.18
Days to maturity	168.95	3.10	166.83	3.91	169.71	2.29	166.86	4.06	160.51	3.51
Plant height (cm)	130.62	16.56	133.47	24.41	135.34	19.70	127.11	12.56	107.00	20.14
Number of pri. Br. per plant	2.33	0.76	3.07	1.05	2.23	0.74	2.03	0.55	2.19	1.07
Number of sec. br. per plant	7.48	0.74	9.20	2.34	7.81	1.15	4.49	1.30	7.20	1.05
Length of main inflores (cm)	35.47	2.31	35.76	5.18	47.66	4.69	39.43	4.28	36.17	2.87
Siliqua length (cm)	3.49	0.38	3.41	0.50	5.49	0.57	3.97	0.33	3.61	0.53
Siliqua width (cm)	0.32	0.04	0.32	0.07	0.48	0.05	0.30	0.03	0.29	0.04
Siliqua len/width ratio	11.20	2.09	11.06	2.84	11.49	1.51	13.40	1.01	12.57	2.05
No. of siliqua per main inflo	20.66	4.01	24.99	4.65	33.80	6.55	24.89	8.49	31.09	6.36
Number of siliqua per plant	191.36	24.58	206.57	37.97	232.57	25.20	131.03	39.05	205.12	31.38
Number of seeds per siliqua	13.84	1.28	14.22	1.48	19.49	2.80	15.80	2.13	13.57	1.97
1000seed wt. (g)	3.25	0.33	2.86	0.33	4.01	0.51	3.71	0.94	2.84	0.29
Seed yield/ plant (g)	8.76	2.66	14.08	5.85	8.47	3.44	5.95	1.50	10.44	4.60
Oil content %	46.17	1.31	45.97	1.51	49.13	1.75	50.13	1.40	48.71	1.68
Protein content %	25.68	0.96	27.47	1.31	24.00	1.49	22.56	1.46	25.11	1.43
Glucosinolates (μMg^{-1})	128.17	6.19	123.01	14.97	103.14	3.72	93.16	14.50	98.38	8.71
Oleic acid %	24.84	1.26	27.38	2.12	33.90	3.54	31.31	6.21	28.84	2.02
Linolenic acid %	10.52	0.76	8.96	0.77	8.26	0.54	8.23	0.43	8.53	0.95
Erucic acid %	53.29	1.38	51.91	1.59	52.87	0.79	45.24	5.49	52.62	1.37

Table 3. Principal components for agro-morphological and seed quality traits in 114 accessions of *Brassica campestris* L. during 2005.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalues	4.946	2.472	2.120	2.006	1.782	1.220	1.014
Cumulative eigenvalues	4.946	7.418	9.538	11.544	13.326	14.546	15.560
Proportion of variance	23.552	11.770	10.097	9.551	8.487	5.809	4.828
Cumulative variance	23.552	35.322	45.419	54.969	63.456	69.265	74.093
Traits							
Days to flower initiation	0.670	0.238	0.048	0.102	0.170	0.080	-0.307
Days to flower completion	0.686	0.466	0.050	-0.071	0.097	0.070	-0.270
Days to maturity	0.530	0.333	0.203	-0.489	0.200	0.249	-0.111
Plant height (cm)	0.519	0.117	0.299	-0.165	0.428	0.076	0.308
No. of primary branches/plant	0.234	0.435	0.066	-0.044	-0.684	0.270	0.192
No. of secondary branches/plant	0.215	0.367	-0.057	-0.113	-0.720	0.348	0.155
Length of main inflorescence (cm)	0.154	-0.288	0.474	0.344	0.182	0.150	0.024
Silique length (cm)	-0.304	0.534	-0.176	0.446	0.403	0.216	0.143
Silique width (cm)	-0.197	-0.563	0.269	-0.045	0.135	0.517	-0.060
Silique length/width ratio	-0.133	0.757	-0.274	0.405	0.267	-0.153	0.175
No. of silique per main inflorescence	0.395	0.0019	0.634	-0.032	0.056	-0.049	0.030
No. of silique per plant	0.420	0.019	0.504	0.459	-0.087	0.020	-0.089
No. of seeds per silique	-0.331	0.225	-0.154	0.319	0.250	0.544	-0.126
1000-seed weight (g)	-0.121	0.253	0.249	-0.662	0.268	0.026	0.329
Seed yield per plant (g)	0.589	0.034	0.153	0.363	-0.189	-0.060	-0.309
Oil contents %	-0.818	0.164	0.095	-0.222	0.032	0.118	-0.252
Protein contents %	0.664	-0.232	-0.110	0.371	-0.044	-0.201	0.389
Glucosinolates (μMg^{-1})	0.820	-0.111	-0.279	-0.134	0.163	0.074	0.193
Oleic acid %	-0.485	0.214	0.622	0.128	-0.003	-0.124	0.206
Linolenic acid %	0.600	-0.119	-0.488	-0.277	0.194	-0.024	-0.157
Erucic acid %	0.146	-0.471	-0.291	0.207	0.042	0.456	0.274

silique per main inflorescence (0.634), number of silique per plant (0.504), 1000 seed weight (0.249), seed yield per plant (0.153) and oleic acid contents (0.622). The PC4 described an additional 9.55% of the total divergence, illustrated primarily the divergence in days to flower initiation (0.102), length of main inflorescence (0.344), silique length (0.446), silique length/width ratio (0.405), number of silique per plant (0.459), number of seeds per silique (0.319), seed yield per plant (0.363), protein contents (0.371), oleic acid contents (0.622) and erucic acid contents (0.207). The PC5 accounted for 8.47% of the total variation and the positive weights towards genetic variation in this component were mainly contributed by days to flower initiation (0.170), days to maturity (0.200), plant height (0.428), length of main inflorescence (0.182), silique length (0.403), silique width (0.135), silique length/width ratio (0.267), number of seeds per silique (0.250), 1000-seed weight (0.268), glucosinolates (0.163) and linolenic acid contents (0.194). The PC6 contributed 5.81% towards genetic variation which was mainly attributed to days to maturity (0.249), number of primary branches per plant (0.270), number of secondary branches per plant (0.348), length of main inflorescence (0.150), silique length (0.216), silique width (0.517), number of seeds per silique (0.544), oil contents (0.118) and erucic acid contents (0.456). The PC7 constituted 4.87% of the total variation and the variation was mainly attributed to plant height (0.308), number of primary branches per plant (0.192), number of secondary branches per plant (0.155), silique length (0.143), silique length/width ratio (0.175), 1000 seed weight (0.329), protein contents (0.389), glucosinolates contents (0.193), oleic acid contents (0.206) and erucic acid contents (0.274).

Table 4. Principal components for agro-morphological and seed quality traits in 114 accessions of *Brassica campestris* L., during 2006.

	PC1	PC2	PC3	PC4	PC5
Eigenvalues	4.957	3.176	2.544	1.755	1.445
Cumulative eigenvalues	4.957	8.133	10.677	12.432	13.877
Proportion of variance	23.606	15.123	12.113	8.357	6.883
Cumulative variance	23.606	38.729	50.842	59.199	66.082
Traits					
Days to flower initiation	0.625	0.065	-0.100	0.358	-0.126
Days to flower completion	0.617	0.206	-0.276	0.488	-0.241
Days to maturity	0.502	0.558	-0.310	0.176	-0.019
Plant height (cm)	0.432	0.527	-0.034	0.275	0.125
No. of primary branches/plant	0.251	0.018	0.530	0.058	-0.353
No. of secondary branches/plant	0.486	0.123	0.609	-0.280	-0.089
Length of main inflorescence (cm)	-0.263	0.563	0.151	0.117	0.237
Silique length (cm)	-0.411	0.647	0.069	0.084	0.397
Silique width (cm)	0.097	0.750	0.170	-0.447	-0.091
Silique length/width ratio	-0.453	-0.201	-0.092	0.561	0.463
No. of silique per main inflorescence	-0.460	-0.051	0.403	0.358	0.335
No. of silique per plant	0.069	0.0912	0.720	-0.010	0.208
No. of seeds per silique	-0.192	0.717	0.117	-0.027	-0.098
1000-seed weight (g)	-0.241	0.692	-0.320	-0.080	0.034
Seed yield per plant	0.195	-0.079	0.570	0.374	0.126
Oil contents %	-0.815	-0.022	-0.109	-0.052	-0.130
Protein contents %	0.714	-0.076	0.487	0.129	0.004
Glucosinolates (μMg^{-1})	0.850	0.122	-0.147	0.0004	0.288
Oleic acid %	-0.600	0.376	0.221	0.159	-0.329
Linolenic acid %	0.590	-0.034	-0.428	-0.186	0.355
Erucic acid %	0.197	-0.109	0.139	-0.556	0.520

Principal component analysis based on fifteen agro-morphological and six seed quality traits during 2006 exhibited that five of the 21 principal components with an eigenvalue higher than one accounted for 66.08% of the total variation among 114 accessions of *Brassica campestris* L. The coefficients defining five principal components of the data are given in Table 4. The PC1 had 21.31% of the total variation in the agro-morphological and seed quality traits. PC1 illustrated primarily the variations in days to flower initiation (0.625), days to flower completion (0.617), days to maturity (0.502), plant height (0.432), number of primary branches per plant (0.251), number of secondary branches per plant (0.486), seed yield per plant (0.195), protein contents (0.714), glucosinolates contents (0.850), linolenic acid contents (0.590) and erucic acid contents (0.197). The PC2 accounted for an additional 15.12% of the total variation and exhibited mainly the patterns of divergence in days to flower completion (0.206), days to maturity (0.558), plant height (0.527), number of secondary branches per plant (0.123), length of main inflorescence (0.563), silique length (0.647), silique width (0.750), number of seeds per silique (0.717), 1000 seed weight (0.692), glucosinolates contents (0.122) and oleic acid contents (0.376). The PC3 contributed 12.11% variation in total variability. The variation in PC3 was mainly attributed to number of primary branches per plant (0.530), number of secondary branches per plant (0.609), length of main inflorescence (0.151),

The results of present studies are in line with those of Elizabeth *et al.* (2001), Choudhry & Joshi (2001) and Alamayeha & Becker (2002). Principal component analysis is useful as it gives information about the groups where certain traits are more important allowing the breeders to conduct specific breeding programs.

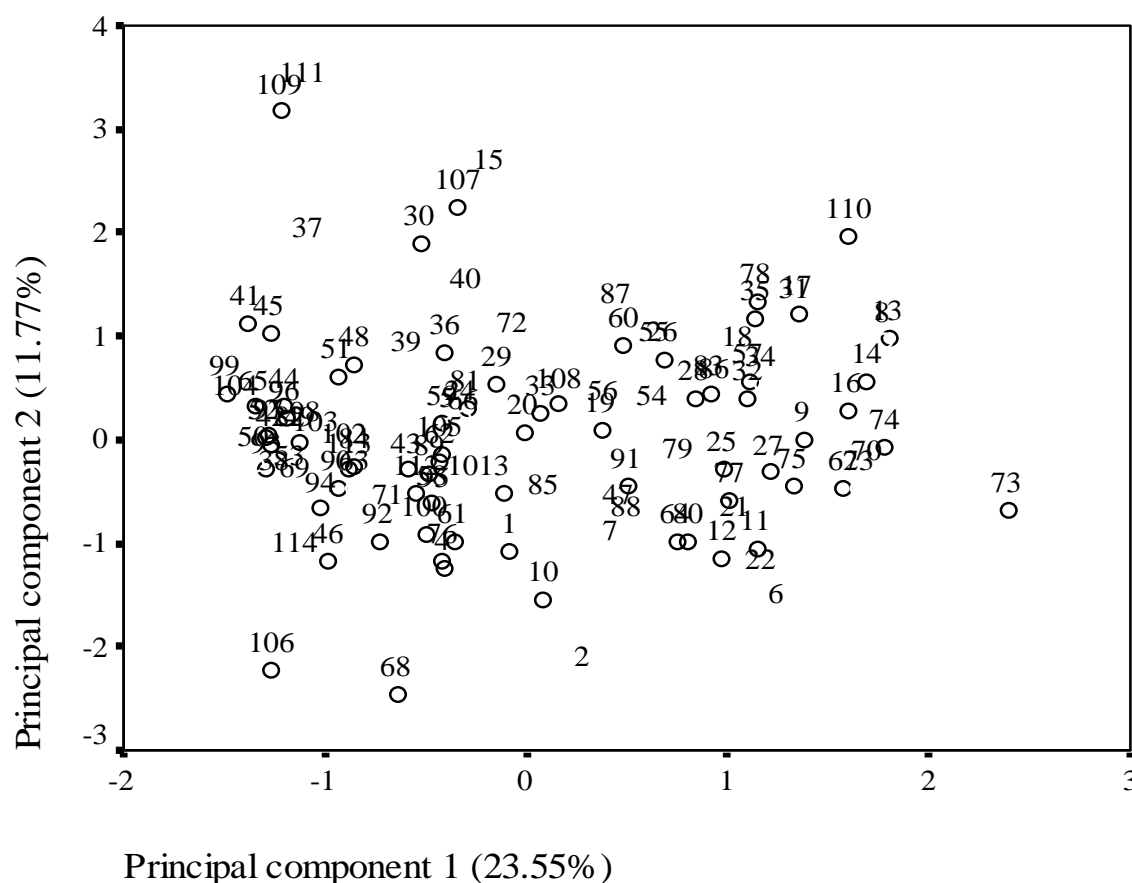


Fig. 1. Scatter diagram for 1st and 2nd PC for fifteen agro-morphological and six seed quality traits in 114 accessions of *Brassica campestris* L. during 2005.

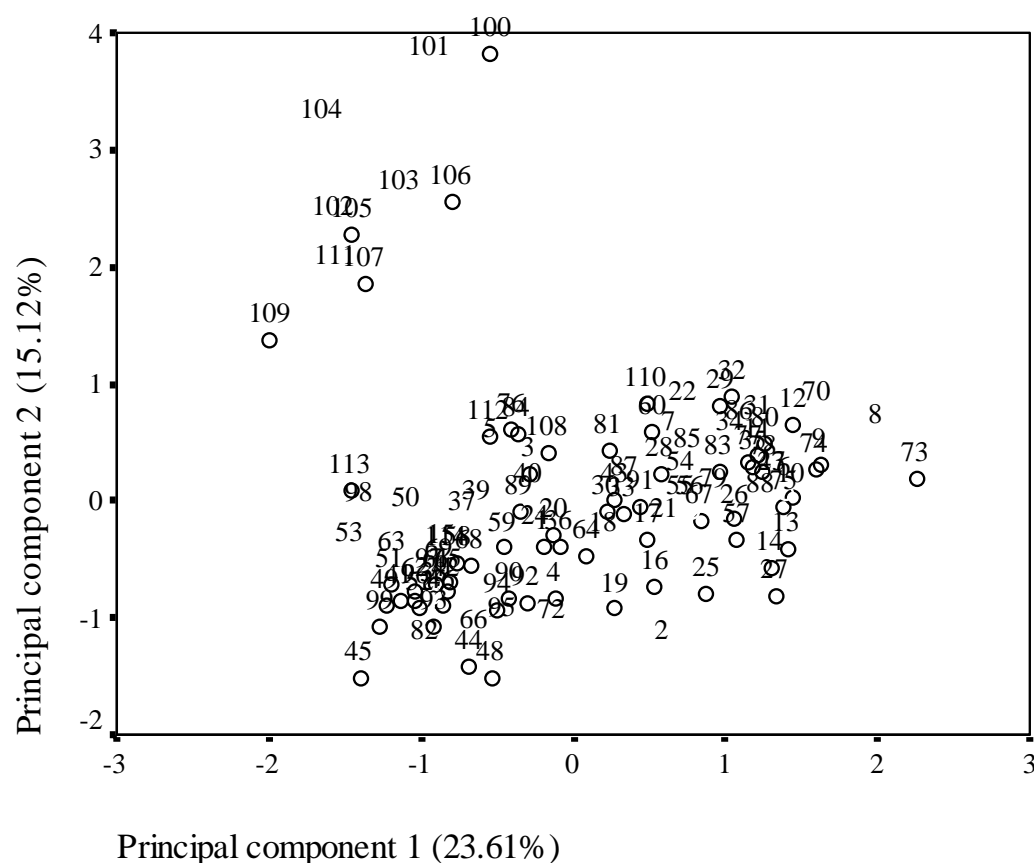


Fig. 2. Scatter diagram for 1st and 2nd PC for fifteen agro-morphological and six seed quality traits in 114 accessions of *Brassica campestris* L. during 2006.

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