GENETIC DIVERSITY AMONG INDUCED MUTANTS OF WINTER BARLEY (*HORDEUM VULGARE L.*)

ГЕНЕТИЧНА ОТДАЛЕЧЕНОСТ МЕЖДУ ИНДУЦИРАНИ МУТАНТИ ЗИМЕН ЕЧЕМИК

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Abstract

The genetic diversity in 40 mutants of barley cultivars Perun and Emon, obtained by single and combination treatments with gamma rays and sodium azide was estimated using two multivariate analyses. A vastly genetic variability for the studied traits between mutants and parent genotypes and as well as among mutants themselves was found. Traits such as plant height, spike length, number of tillers/1m^2, winter hardiness and 1000 grain weight have major contributions to the induced genetic diversity. The most promising genotypes for future hybridisation program were mutants M48, M27 and M30.
Keywords: barley, genetic diversity, multivariate analyses, mutants

Резюме

Оценена е генетичната отдалеченост на 40 мутанти, получени от сортовете Перун и Емон след самостоятелно и комбинирано третиране с гама-лъчи и натриев азид чрез прилагане на два мултивариантни метода за анализ. Наблюдавано е значително генетично разнообразие между мутантите и между мутантите и изходния сорт по проучваните признаци. Установено е, че признаците, които допринасят най-много за генетичната отдалеченост сред мутантите са височина на растенията, дължина на класа, брой класоносни стъбла/1m^2, зимоустойчивост и маса на 1000 зърна. Най-подходящи за използване в бъдещата хибридизационна програма са мутантите М48, М27 и М30.
Ключови думи: ечемик, генетична отдалеченост, мултивариантни анализи, мутанти

Разширено резюме

Проучването беше проведено в опитното поле на Институт по земеделие-Карнобат през периода 2006/2007-2007/2008 година. В изследването бяха включени 40 мутанти, получени от сортовете Перун и Емон чрез самостоятелно и комбинирано третиране с гама-лъчи и натриев азид. Мутантите и изходните сортове бяха засявани на площ 1m^2 в три повторения. При полски условия бяха определени височина на растенията, зимоустойчивост, дата на изкласяване,

чувствителност към брашнеста мана- *Erysiphe graminis* и ленточна болест- *Helminthosporium gramineum*, брой класоносни стъбла/1m^2 . Върху 10 рандомизирано взети растения от всеки генотип и от всяко повторение бяха отчетени: дължина на класа, дължина на осила, брой класчета в клас, брой зърна в клас, тегло на зърното от клас и маса на 1000 зърна. Генетичната отдалеченост беше оценена с помощта на два мултивариантни метода за анализ – анализ на главните компоненти и кластерен анализ. И двата използвани метода за оценка на колекцията от мутанти демонстрираха, че в резултат на индуцирания мутагенезис е създадено генетично разнообразие по проучваните признаци. Установено беше, че признаците, които допринасят най-много за генетичната отдалеченост сред мутантите са височина на растенията, дължина на клас, брой класоносни стъбла/1m^2, зимоустойчивост и маса на 1000 зърна. Извършеното групиране на мутантите в нашето проучване ще улесни използване им в комбинативната селекция. Поради добро съчетание на агрономически важни признаци мутантите M48, M27 и M30 бяха определени като подходящи за включване в селекционната програма.

Introduction

Experimental mutagenesis is used to generate additional genetic variability for crop improvement. In mutation breeding programs it is a common experience that mutations result in a spectrum of phenotypic changes, due to the pleictropic action of the mutated genes or the additional mutational effects on an individual plant. Univariate analysis would not be adequate to distinguish the mutants with many differences in the agronomic traits. Alternatively, multivariate analysis which takes into consideration several traits simultaneously would be a dependable method in determining differences among the induced mutants. Use of multivariate analysis for identification and classification of mutants has earlier been reported in rice (Chandra et al. 2007), finger millet (Muduli and Misra, 2008), grasspea (Kumar and Dubey, 2003).

The present study was undertaken with objectives: a) to assess and evaluate genetic diversity of barley mutants; b) to identify characters which contribute maximum to genetic diversity and c) to identify suitable mutants for future use in breeding programs in barley.

Material and method

This research was conducted in the 2006-2007 and 2007-2008 growing seasons in the experimental field of the Institute of Agriculture – Karnobat, Southeastern Bulgaria.

The soil of experimental field was slightly acid (pH is 6.2) leached chernozem-smolniza. Long term average precipitation for this region was 422.1 mm per growing season. The amount of precipitation in the first year growing period was much lower (277.8 mm) than that in the second year (509.5 mm).

As plant materials, mutants derived from the varieties Perun (Navushtanov, 1997) and Emon (Mersinkov, 2003) of barley (*Hordeum sativum L*.) were used. Perun and Emon are widely grown in Bulgaria winter two-rowed malting barley varieties developed by the Institute of Agriculture – Karnobat.

Seeds of two varieties were submitted to single and combined treatments with gamma rays and sodium azide. Irradiation was applied on air-dry seeds with dose

100, 200, 300 Gy gamma rays ^{60}Co. For chemical mutagens treatment, seeds were presoaked in distilled water for 16 hours followed by treatment in freshly prepared 1.0 and 2.0 mM solutions of sodium azide at pH 3.0 for 2 hrs at 25 ± 2^{o}C. Immediately after treatments, the seeds were thoroughly washed in tap water. In combined treatment immediately after irradiation with 100 Gy seeds were presoaked for 16 hours and treated with 1.0 mM sodium azide.

M_1 plants grown after mutagenic treatments were propagated based on the spike progeny method. The M_2 seeds obtained from the main-stem spike were sown to rows. Selection of mutants was carried out in the M_2 and M_3 generations. M_2 plants showing a difference from the control and plants with desired phenotypes were harvested individually. Then M_3 progeny from selected M_2 plants according to the pedigree selection procedure were grown. The mutants with desired changes were transferred to the M_4 generation.

In M_5 and M_6 generations selected stable mutants were evaluated for 12 traits (Table 1). The traits not scored in the field (SL, AL, SN, GN, GW, TGW) were measured in the laboratory on ten plants sampled from the middle of the plot of each genotype in each replication. Heading period was determined as day between the emergence of plants date and the heading date of 75% of the plants in the plot. Susceptibility to *Erysiphe graminis f. sp. hordei* and *Helminthosporium gramineum* were measured according to an scale from 1, fully resistance – plants are without visible symptoms of infection, to 9, the highest susceptibility – extreme infection on entire plants. A 1-9 scale was used for scoring winter hardiness 1- very low (below 60% survival plants), to 9 – very high (above 95% survival plants after winter).

The experiments were organized in a Randomized Complete Block Design with 3 replications. Each plot consisted of five 120 cm rows, 20 cm apart. Sowing was performed by hand on October 22, 2006, in the first year of the trial and on October 25, 2007, in the second year. Standard agronomic and plant protection practices were used.

Two multivariate analyses - principal component and cluster analyses were utilized for the evaluation of mutants. Principal component were obtained by SPSS 12.00 for Windows. The cluster analysis was performed using the program Statistica that adopts Euclidian distance as a measure of dissimilarity and the Ward's method as the clustering algorithm (Ward, 1963). Before computing our data were standardized.

Table1. Name and abbreviations of agronomic characteristics evaluated in winter barley mutants

Characters	Codes
Spike length (cm)	SL
Awn length (cm)	AL
Spikelet number per a spike	SN
Grain number per a spike	GN
Grain weight per a spike (g)	GW
1000 grains weight (g)	TGW
Plant height (cm)	PH
Days to heading	DH
Susceptibility to *Erysiphe graminis* (scale 9-1)	EG
Susceptibility to *Helminthosporium gramineum* (scale 9-1)	HG
Winter hardiness (scale 1-9)	WH
Spike number per 1m^2	NS

Results and discussion

Large variability was observed for most of the characters studied (Table 2). According to the type and density of the spike, studied two-rowed barley mutants might divide into three botanical varieties: *var. erectum* (11 mutants derived from the cultivar Emon), *var. nutans* (10 mutants derived from the cultivar Perun and 7 from Emon), *var. zeocritum* (8 mutants derived from the cultivar Emon). Four six-rowed mutants - M57, M58 и M59 from Emon and M28 from Perun were also included in this study.

Spike length ranged from 4.19 to 11.67 cm with coefficient of variability of 11.67 per cent. Awn length varied from 2.35 to 18.40 cm. Spikelet number per a spike varied from 25.42 to 80.12 cm with a CV 37.73 per cent. Grain number per a spike ranged from 19.01 to 60.73 and CV was 27.69 per cent. Grain weight per a spike varied from 0.82 to 2.07 g and with a CV of 18.62 per cent. Weight of 1000 grains ranged from 27.25 to 55.15 g in the mutants evaluated. The CV observed was 15.77 per cent. Plant height varied from 51.0 to 104.0 cm and with a CV of 14.14 per cent. Days to heading ranged from 186 to 196 days and CV was 21.14 per cent. Susceptibility to powdery mildew *(Erysiphe graminis f. sp. hordei)* showed a wide range of 1 to 8 score with a CV of 51.45. Wide variation in susceptibility to barley stripe (*Helminthosporium gramineum*) showed different barley mutants. Maximum CV (63.47 %) was observed for this character. Winter hardiness ranged from 9 to 1 score with a CV of 42.28 per cent. Spike number per $1m^2$ varied from 286 to 792 and with a CV of 26.33 per cent.

The variation studied through Principal Component Analysis revealed that three principal components having greater than 1 eigenvalues contributed 71% of the total variation (Table 3). It was found that Principal Component 1 (PC1) contributed 34.98%, whereas PC2, PC3 contributed 23.54% and 12.63% respectively of the total variation. The traits, which contributed positively to PC1 were plant height (0.787), spike length (0.739), number of tillers/$1m^2$ (0.729) winter hardiness (0.677), 1000 grain weight (0.623) and grain weight per a spike (0.567). Heading days and resistance to powdery mildew contributed negatively to the first component. Maximum genetic variance to PC2 was contributed by number of spiklets and grains per spike. 1000 grain weight had a negative contribution for this component. In the case of PC3, grain weight per spike contributed positively and resistance to *Helminthosporium gramineum* had a negative contribution.

Figure 1 displays a biplot in the dimension of the first and second PCs. Parent variety Emon and two poly-rowed mutants - M58 and M59, derived of this variety had positive values for PC1 and PC2. Most of mutants derived from Emon (M35, M29, M33, M34, M32, M36, M37, M39), belong to var. *erectum* and almost all of the mutants of var. *zeocritum* (M49, M50, M51, M52, M53, M55) had negative values for first and second PCs. Almost all mutants from var. *nutans* had positive values for PC1 and negative values for PC2. Positive values for PC2 and negative values for PC1 had 6 mutants (M54, M31, M41, M38, M28 и M57).

To have a clear idea of the patterns of variation in the traits correlated with the third principal component (grain weight per spike and resistance to *Helminthosporium gramineum*) in relation to the differences in the agronomic traits correlated with the first principal component, the distribution of the mutants was plotted along the axes of the first and the third principal components (Figure 2). All mutants from the var. *zeocritum* had negative values for PC3. Highest positive value for PC3 had a M57- a

Table 2. Mean values of 12 agronomic characters of barley mutants, derived from cultivars Perun and Emon (2007-2008)

Genotypes	Characters											
	SL	AL	SN	GN	GW	TGW	PH	DH	EG	HG	WH	NS
Perun	9.14	16.43	26.78	23.66	1.48	48.48	101.0	190	2	3	8	692
M20	9.75	17.67	28.03	25.75	1.36	50.45	97.0	188	2	3	7	656
M21	8.21	11.23	27.07	24.00	1.27	55.13	100.0	188	2	3	7	645
M22	9.29	15.18	28.63	25.48	1.32	46.20	102.5	190	4	7	5	712
M23	9.76	17.17	30.90	28.66	1.58	43.95	88.0	191	3	3	6	499
M24	9.11	17.11	27.75	25.30	1.39	48.50	93.5	189	8	1	3	664
M25	8.51	10.24	27.63	24.11	1.21	46.08	96.0	190	5	3	3	752
M26	10.47	14.48	27.16	23.83	1.18	50.03	104.0	186	2	1	7	688
M27	11.67	18.06	30.94	28.73	1.59	50.45	97.5	190	1	1	7	668
M28	8.80	2.35	69.69	32.81	1.23	29.60	94.0	193	8	3	7	580
M29	9.70	17.73	30.26	28.44	1.58	47.03	82.0	196	5	3	1	460
M30	11.49	17.77	30.93	28.80	1.62	54.38	104.5	188	1	3	5	792
Emon	7.16	15.68	32.95	31.10	1.53	43.15	94.0	190	3	3	9	700
M31	7.03	13.73	34.74	31.86	1.27	37.05	78.0	192	5	3	5	572
M32	7.00	14.77	29.36	28.68	1.24	39.75	97.0	190	4	3	7	388
M33	7.66	15.69	33.73	31.53	1.51	43.80	96.0	196	8	1	3	286
M34	7.86	15.23	29.23	27.50	1.24	39.70	101.0	190	8	3	3	652
M35	6.99	15.68	32.53	29.46	1.52	45.25	92.0	192	8	1	6	380
M36	7.19	12.67	26.96	20.37	0.94	41.03	79.0	192	8	7	3	580
M37	7.47	9.70	32.00	28.80	1.41	41.25	104.0	193	8	1	3	316
M38	7.84	15.15	39.25	31.67	1.48	40.38	87.0	192	8	3	3	700
M39	7.54	10.09	32.45	28.90	1.37	40.00	87.0	193	8	1	3	316
M40	7.37	16.37	28.79	24.76	1.42	46.25	90.0	190	5	5	5	452
M41	6.21	17.14	34.76	32.35	1.77	45.00	87.0	192	8	3	7	380
M42	9.94	16.11	29.92	28.07	1.30	39.88	97.0	190	8	3	3	620
M43	9.23	16.59	33.04	30.45	1.50	45.28	92.0	190	3	1	7	684
M44	10.19	18.40	31.72	29.94	1.48	47.95	94.0	190	3	3	9	676
M45	10.13	15.27	26.82	24.88	1.55	55.15	86.0	191	2	1	7	636
M46	10.14	17.68	29.79	25.95	1.46	45.42	97.0	192	1	3	7	700
M47	9.30	17.31	31.53	27.58	1.52	50.50	97.0	189	5	7	5	724
M48	9.29	17.82	30.53	30.29	1.69	48.13	91.0	193	8	3	5	676
M49	4.19	12.82	25.43	19.01	0.81	36.55	62.0	195	8	1	1	300
M50	5.12	15.07	32.85	25.46	0.92	31.83	52.0	193	8	1	3	364
M51	5.14	14.95	33.11	21.16	0.91	34.55	51.0	192	8	3	1	348
M52	4.32	15.29	27.71	21.33	1.09	41.00	76.0	194	8	3	7	684
M53	4.93	13.46	29.34	23.12	0.94	36.43	74.0	190	6	5	3	316
M54	5.25	14.37	31.78	27.06	1.39	41.70	84.0	193	8	5	7	700
M55	4.49	12.61	27.67	23.99	1.07	39.35	79.0	193	4	3	7	580
M56	5.44	14.52	31.96	28.73	1.35	41.54	96.0	191	3	5	7	716
M57	7.02	13.60	63.06	56.43	2.06	38.30	79.0	192	8	1	1	580
M58	6.12	11.82	77.53	60.73	1.71	27.25	97.0	188	2	7	8	612
M59	6.28	13.76	80.12	45.03	1.60	28.30	99.0	189	2	9	7	740
Min	*4.19*	*2.35*	*25.42*	*19.01*	*0.82*	*27.25*	*51.0*	*186*	*1*	*1*	*1*	*286*
Max	*11.67*	*18.40*	*80.12*	*60.73*	*2.07*	*55.15*	*104.0*	*196*	*8*	*9*	*9*	*792*
CV%	*25.49*	*20.31*	*37.73*	*27.69*	*18.62*	*15.77*	*14.14*	*1.14*	*51.45*	*63.47*	*42.28*	*26.33*

Table 3. Principal Component analysis of barley mutants, derived from cultivars Perun and Emon

Characters	PC1	PC2	PC3
Eigenvalues	4.197	2.824	1.515
Proportion of variance	34.976	23.534	12.628
Cumulative variance	34.976	58.510	71.138
	Factor loadings		
SL	0.739	-0.276	0.314
AL	0.406	-0.437	0.244
SN	0.002	0.959	0.116
GN	0.167	0.861	0.410
GW	0.567	0.353	0.681
TGW	0.623	-0.684	0.175
PH	0.787	0.111	0.075
DH	-0.706	-0.105	0.355
EG	-0.778	-0.026	0.259
HG	0.189	0.491	-0.566
WH	0.677	0.159	-0.322
NS	0.729	0.109	-0.242

multi-rowed mutant with high grain weight per a spike. High positive values for PC1 and PC3 had M27 and M30, derived from Perun and M48 from Emon. This mutants could be use in yield improvement, as a combination of high grain weight per a spike and high spike number per $1m^2$ in one genotype is desirable in barley breeding.

The dendrogram of the evaluated barley mutants is presented in Figure 3. Forty mutants and their parent varieties were grouped into four clusters at a distance of 15 units. Cluster I included four multi-rowed mutants - M59, M58, M57 derived from Emon and M28 from Perun. Claster II has the tree mutants belong to var. *zeocritum* having short spike, short stature and low productivity. The Claster III which comprised 23 mutants and parent variety Emon was the largest cluster. Cluster IV consisted 10 mutants – M26, M21, M29, M45, M27, M43, M30, M46, M44, M20 and parent variety Perun belonging to the *var. nutans*. This cluster had mutants with maximum in weight of 1000 grains, spike number per $1m^2$, field resistance to *Erysiphe graminis* and *Helminthosporium gramineum*, winter hardiness (Table 4).

Observations from cluster mean suggested that none of the clusters contained genotypes with all the desirable traits, which could be directly selected and utilized. Interestingly, most of the minimum and maximum cluster means were distributed in relatively distant clusters. The hybridisation between genotypes of different clusters is necessary for the development of desirable genotypes. Recombination breeding between genotypes of different clusters has also been suggested by Sharma et al. (1998) and Bose and Pradhan (2005). Members of cluster having high genetic distance might be produce superior segregants. The production of transgressive variants by hybridization of mutants has earlier been reported (Maluszynski et al., 1991; Misra, 1995).

Separation of mutants from parent cultivars shows effect of used mutagens in creating genetic diversity. All results mentioned suggest that the diversity present in this mutant barley collection represents a good base for improving existing barley cultivars for some individual traits. Using induction mutation, similar improvements in

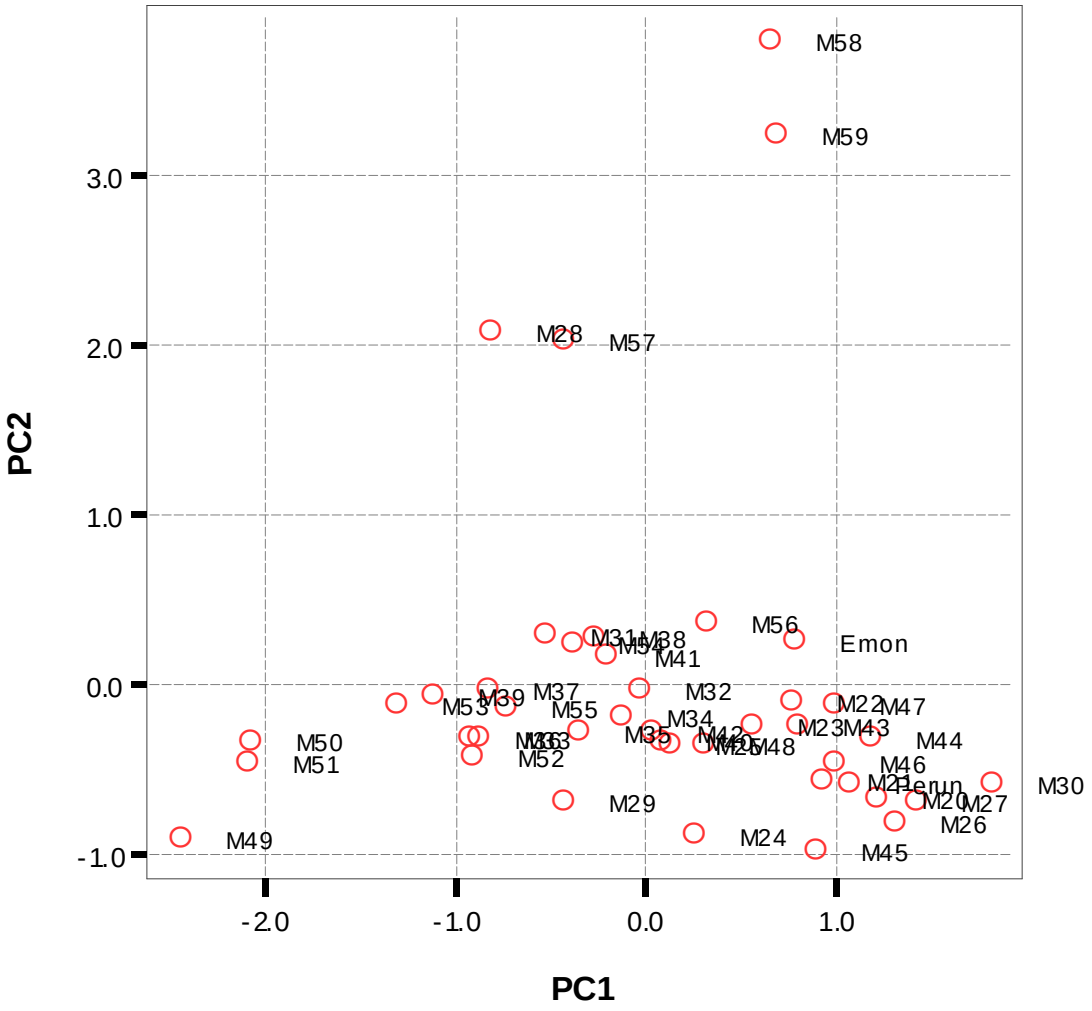

Figure1. Scatter diagram for PC1 and PC2 in 40 barley mutants

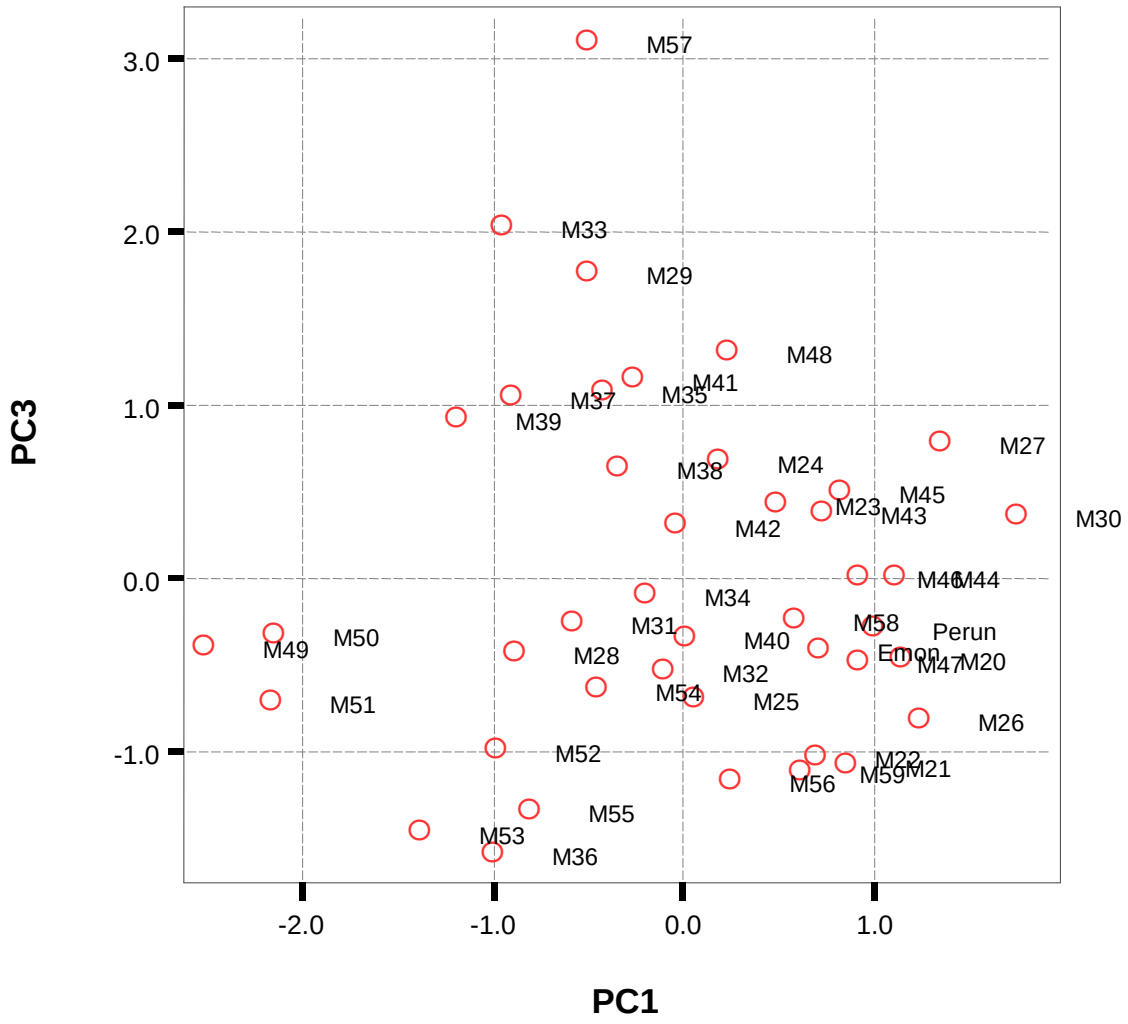

Figure 2. Scatter diagram for PC1 and PC3 in 40 barley mutants

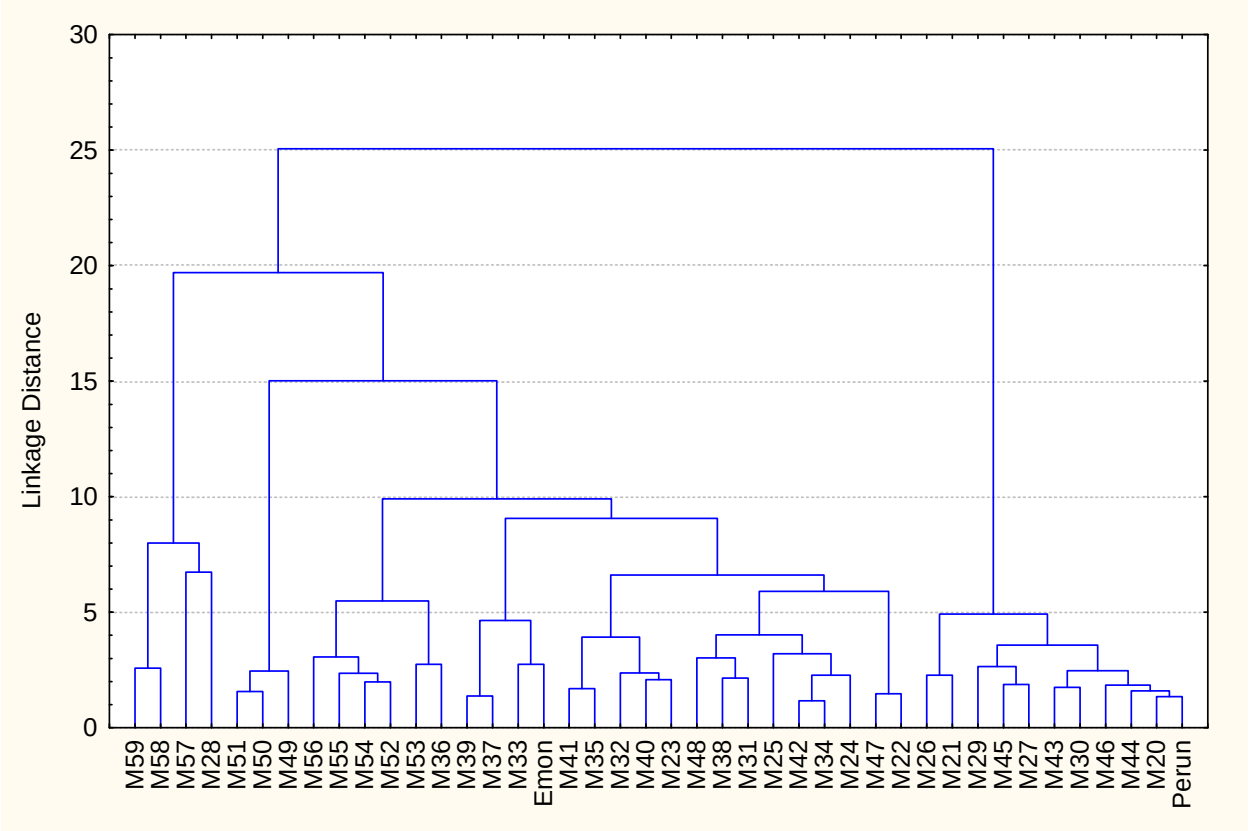

Figure 3. Dendrogram base on 12 agronomic traits of barley mutants derived from cultivars Perun and Emon (2007-2008)

Table 4. Cluster mean of 12 agronomic characters of barley mutants, derived from cultivars Perun and Emon (2007-2008)

Characters	Cluster			
	I /4/*	II /3/	III /24/	V/11/
Spike length (cm)	7.05	4.82	7.37	10.02
Awn length (cm)	10.38	14.28	14.73	16.43
Spikelet number per a spike	72.60	30.46	30.89	29.38
Grain number per a spike	48.75	21.88	27.52	26.78
Grain weight per a spike (g)	1.65	0.88	1.36	1.46
1000 grains weight (g)	30.86	34.31	42.87	49.69
Plant height (cm)	92.25	55.00	90.00	96.45
Days to heading	190.50	193.33	191.67	189.45
Susceptibility to *Erysiphe graminis* (scale 9-1)	5.00	8.00	6.38	2.00
Susceptibility to *Helminthosporium gramineum* (scale 9-1)	5.00	1.67	3.42	2.27
Winter hardiness (scale 1-9)	5.75	1.33	5.04	7.18
Spike number per $1m^2$	628.00	337.33	555.25	666.91

* Figures in parentheses indicate number genotypes included in cluster

the agronomic characters of barley were reported (Gomez-Pando et al., 2009; Lundqvist, 2009; Molina-Cano et al., 2003).

Among 12 traits under study, a considerable diversity was observed for plant height, spike length, number of tillers/1m^2, winter hardiness and 1000 grain weight. Chand et al. (2008) reported high diversity for number of grains per spike and grain yield per plant in elite barley lines. At the same time, Abebe et al. (2008) studied the diversity of the Ethiopian barley germplasm through morphological traits and found a considerable diversity for days to heading, days to maturity, biomass, plant height and 1000-grain weight.

Of particular interest for barley breeding are mutants with improve winter hardiness and disease resistance. Resistance to PM, HG and higher winter hardiness needs to be reconfirmed. Additional tests are required to determine the number of resistance genes present in the mutants and to study their allelism to known genes.

Conclusions

The multivariate methods applied revealed considerable genetic diversity for important agronomic traits of mutants derived from the winter barley cultivars Perun and Emon. Plant height, spike length, number of tillers/1m^2, winter hardiness and 1000 grain weight were observed to be the major contributors to the genetic diversity. Considering agronomic performance the mutants M48, M27 and M30 these genotypes might be selected as promising genotypes for future hybridization program.

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