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Grain yield and yield related traits of sodium azide induced barley mutant lines

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Добив от зърно и свързани с добива признаци при индуцирани с натриев азид мутантни линии ечемик

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ABSTRACT

The aim of this study was to evaluate grain yield and yield related traits of sodium azide induced mutant lines from the winter barley variety Kuber. Eighteen advanced mutant lines, parent variety and national standard varieties Obzor and Emon were evaluated. The research was conducted through three seasons (from 2014/2015 to 2016/2017) in the experimental field of the Institute of Agriculture – Karnobat, South- eastern Bulgaria. Highly significant differences among mutants for days to heading, plant height, number of spikes per plant, awn length, peduncle length, spikelet number per spike, grain number per spike, grain weight per spike, 1000 grain weight and grain yield were found. Mutant lines 16/1-3, 16/1-8, 16/1-21, 16/2-7, 16/2-20 and 16/3-12 had a significantly higher grain yield over parent Kuber and over standards Obzor and Emon. Grain yield had a significant positive correlation with the number of spikes per plant and spike length. The maximum phenotypic and genotypic variability were observed for number of spikes per plant and spike length. Spikelet and grain number per spike showed high heritability coupled with high genetic advance, suggesting that these traits could be improved more easily by selection.

Keywords: barley, grain yield, mutation, sodium azide

РЕЗЮМЕ

Целта на това изследване е да се оцени добива от зърно и свързани с добива признаци при мутантни линии, индуцирани с натриев азид от зимен ечемик сорт Кубер. Осемнадесет перспективни мутантни линии, изходния сорт и националните стандарти - сортовете Обзор и Емон са изпитвани по блоков метод в 4 повторения. Опитът е изведен в експерименталното поле на Институт по земеделие - Карнобат, Югоизточна България през периода от 2014/2015 до 2016/2017 г. Установени са доказани различия между мутантите и изходния сорт по признаците дни до изкласяване, височина на растенията, брой класове на растение, дължина на осила, дължина на последното междувъзлие, брой класчета в клас, брой зърна в клас, тегло на зърното от клас, тегло на 1000- зърна и добив от зърно. Доказано по-висок добив спрямо изходния сорт Кубер и стандартите Обзор и Емон е получен от мутантните линии 16/1-3, 16/1-8, 16/1- 21, 16/2-7, 16/2-20 и 16/3-12. Добивът от зърно е в доказана положителна корелация с броя на класовете в растение и с дължината на класа. Максимални стойности на фенотипния и генотипния коефициент на вариране са отчетени за признаците брой на класовете в растение и дължина на класа. Висока наследяемост, съчетана с висок генетичен напредък е установена за броя на класчетата и зърната в класа, което предполага, че тези признаци могат да бъдат подобрени сравнително лесно.

Ключови думи: ечемик, мутация, добив на зърно, натриев азид

INTRODUCTION

Barley is one of the major grain crops of the world, cultivated successfully in a wide range of climatic conditions. One of the main aims of any breeding program is to produce high-yielding and better-quality lines for release as varieties. The prerequisite to achieve this goal is to find a sufficient amount of variability, in which desired lines can be selected for further manipulation to achieve the target.

Different breeding techniques as conventional and wide hybridization, genetic engineering and molecular-marker assisted breeding are being used successfully to improve yield and other traits in barley. Mutation breeding is one of the important techniques to induce genetic variation.

The induced mutations had significantly contributed to developing superior varieties of many crops. Those mutant varieties had a great economic impact on agriculture and food production in many countries, including Bulgaria (Ahloowali et al., 2004; Tomlekova, 2010). More than 3000 varieties of different crops have been officially released by the mutation breeding technique. The mutants developed in barley have great potential for direct release and to include them in cross breeding programmes. Many barley cultivars developed in the world through induced mutation have been released which possess tolerance to biotic and abiotic stresses and improved yield (Ahloowali et al., 2004).

Ionizing radiations constitute the most commonly used physical mutagens (Mba, 2013). Besides ionizing radiations, chemical mutagens like ethylnitroso-urea, methylnitroso-urea, ethylmethane-sulphonate (EMS) and sodium azide (SA) are also widely used for mutation breeding. Among chemical mutagens, sodium azide is considered as relatively safe and has the capability to produce a high frequency of mutation (Kleinhofs et al., 1978). This mutagen has been used in various crops to improve their yield, quality traits and resistance against biotic and abiotic stresses (Khan et al., 2009).

The aim of this study was to evaluate grain yield and yield related traits of sodium azide induced mutant lines from winter barley variety Kuber.

MATERIAL AND METHODS

The study was conducted in three growing seasons from 2014/2015 to 2016/2017 on the experimental field of the Institute of Agriculture - Karnobat, South-eastern Bulgaria (42°39' N, 26°59' E). The field experiment was set up on leached chernozem soil under rainfed conditions. The experiment was organized in a Randomized Complete Block Design with 4 replications on plots of 10 m2. Standard agricultural and plant protection practices were applied.

The plant material consisted of 18 mutant lines derived from variety Kuber, parent variety and national standard varieties Obzor and Emon. Variety Kuber is a winter high-yielding malting 2-rowed barley variety, developed at the Institute of Agriculture- Karnobat (Gocheva and Vulchev, 2014).

For mutation induction seeds were pre-soaked for 16 hours and were treated with 2 mM sodium azide for 2 hours, prepared in a buffer solution (pH=3) at room temperature. The M1 generation plants grown in the field were harvested in bulk. In M2 progeny 200 plants with the highest grain weight per main spike were selected. From M3 to M6 all mutants with a lower grain yield compared to parent variety or with other undesirable agronomical characteristics were discarded. Selected mutant lines were included in the present investigation.

The studied traits included days to heading (DH), plant height (PH, cm), number of spikes per plant (SP), spike length (SL, cm), awn length (AL, cm), peduncle length (PL, cm), spikelet number per spike (SNS), grain number per spike (GNS), grain weight per spike (GWS, g), 1000 grain weight (TGW, g), grain yield (GY, $t*ha^{-1}$).

The data were recorded on plant basis by randomly selecting 10 plants from each plot. Days for heading, 1000 grain weight and grain yield were estimated on a plot basis.

Heritability in broad sense (h^2) was estimated according to Falconer (1989):

$$h^2 = (\sigma^2 g) / (\sigma^2 ph)$$

where:

$\sigma^2 g$ - genotypic variance,
$\sigma^2 ph$ - phenotypic variance.

Genotypic ($\sigma^2 g$) and Phenotypic variances ($\sigma^2 ph$) were obtained from the analysis of variance table according to Comstock and Robinson (1952).

The mean values were used for genetic analyses to determine Phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of Variation (GCV), according to Singh and Chaudhury (1985):

$$GCV\ (\%) = ((\sqrt{\sigma^2 g}) / x) * 100$$

$$PCV\ (\%) = ((\sqrt{\sigma^2 ph}) / x) * 100$$

where:

$\sigma^2 g$ - genotypic variance,
$\sigma^2 ph$ - phenotypic variance,
X - sample mean.

Genetic advance (GA) was calculated with the method suggested by Allard (1960) and Singh and Chaudhury (1985):

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

where:

K - constant = 2.06 at 5% selection intensity,
$\sigma^2 ph$ - phenotypic variance,
h^2- heritability in broad sense.

Analysis of variance and the correlations (Person's correlation coefficients) were computed for all the traits evaluated using the computer software system of SPSS 16.00 for Windows 16.0 (SPSS Inc., 2007).

RESULTS AND DISCUSSION

The analysis of variance revealed significant differences for genotypes and years for all the studied traits (Table 1). Effect of genotype by environment interaction was significant for days to heading, plant height, number of spikes per plant, spike length, spikelet number per spike, grain number per spike, grain weight per spike, 1000 grain weight and grain yield.

The percentages of the total sums of squares accounted for by genotype, year, and genotype by year interaction have been used for a showing of the variation attributed to each component. The contribution of the factor „year" was highest for plant height (87.21%), followed by grain number per spike (72.51%), 1000 grain weight (67.40%), days to heading (53.77%) and spikelet number per spike (52.84%). The percent of genotype from the total sum of squares was maximum for spike length (74.11%), awn length (77.11%) and number of spikes per plant (59.70%). The magnitude of the sum of squares explained by genotype by year interaction was near to those of genotype and environment for grain weight per spike and grain yield. Significant genotype by year interaction effects for the most traits indicated that substantial differences in genotypic response across different years. Previous studies have also reported significant genotype by environment interaction effect for grain yield and most of the yield related traits in barley (Kadi et al. 2010; Bleidere et al. 2012; Ebadi- Segherloo et al. 2016).

Mean of studied traits of the mutant lines, parent and check varieties (2014/2015 – 2016/2017) are presented in Table 2 and Table 3. There were significant differences in the number of days to heading between parent (192.33) and mutant lines 16/1-1 (194.17) and 16/2-20 (190.50). Plant height of mutant lines varied from 74.41 cm to 91.88 cm. Line16/2-20 had a significantly lower plant height compared with the parent. Three of mutant lines 16/1-3, 16/2-20 and 16/3-12 had significantly more spikes per plant than variety Kuber and standard varieties. There were no significant differences between mutant lines and parent variety in spike length. Awn length ranged from 8.42 cm to 11.08 cm among the studied lines. Peduncle length of the mutant lines varied from 15.88 cm (16/3-8) to 20.67 cm (16/1-16), while that for the parent line was 18.75 cm. Spikelet number per spike ranged from 26.17 to 33.05. Lines 16/1-1, 16/2-7 16/2-19, 16/2-23 and 16/3-12 had significantly more spikelets per spike compared with the parent variety Kuber. Mutant lines with higher (16/2-7, 16/2-19 and 16/3-12) and lower (16/1-14, 16/1-16, 16/1-2 and 16/3-8) grain number per spike than the parent were found. The highest grain weight per spike (1.60 g) was observed in 16/1-1, followed by 16/1-8 (1.59 g), 16/1-10 (1.58 g) and 16/2-7 (1.58 g).

The mean value of 1000 grain weight of the lines 16/1-4, 16/1-13, 16/1-16, 16/2-20, 16/3-12 and 16/3-14 was lower compared to Kuber. The grain yield of mutant lines varied from 6.39 t*ha^{-1} to 7.23 t*ha^{-1}. Lines 16/1-3 (7.15 t*ha^{-1}), 16/1-8 (7.05 t*ha^{-1}), 16/1-21 (7.23 t*ha^{-1}), 16/2-7 (7.14 t*ha^{-1}), 16/2-20 (6.99 t*ha^{-1}) and 16/3- 12 (6.98 t*ha^{-1}) had a significantly higher grain yield over parent Kuber (6.56 t*ha^{-1}) and over standards Obzor (5.08 t*ha^{-1}) and Emon (6.14 t*ha^{-1}).

The correlations between grain yield and yield related traits for mutant lines are presented in Table 4. Grain yield exhibited a significant and positive correlation with the number of spikes per plant (*r*=0.534) and spike length (*r*=0.495). The similar significant positive association among yield and spike length in barley was also observed by Budakli Carpici and Celik (2012). The correlation between plant height and grain yield was negative, but not significant (*r*=-0.206). Plant height had positive correlation with 1000 grain weight (*r*=0.497) and negative correlation with the number of spikes per plant (*r*=-0.469). Awn length had significant correlation with spikelet and grain number per spike (*r*=0.653 and *r*=0.635) and grain weight per spike (*r*=0.566). Some authors reported for correlation between peduncle length and grain yield in barley (Jouyban et al., 2015; Lodhi et al., 2015; Singh et al., 2015) but in this study that correlation was not significant. Significant positive correlations were determined between spikelet number per spike and grain number per spike (*r*=0.947) and spikelet number per spike and grain weight per spike (*r*=0.626). Grain number per spike showed a significant positive relationship with grain weight per spike (*r*=0.691). The relationship between spike length and grain number per spike is indicative of simultaneous genetic improvement in these two traits. Several researchers like Gocheva (2014), Budakli Carpici and Celik (2012) have also reported positive association between spike length and grain number per spike in barley. The 1000 grain weight and grain yield were non-significantly related. Similar results were reported by Drikvand et al. (2011) and Budakli Carpici and Celik (2012), while Al-Tabbal and Al-Fraihat (2012) and Gocheva et al. (2017) have reported that weight of 1000 grain was

Table 1. Mean squares (MS) and percent contribution of sum of of squares of each component to total sum of squares (SS,%) for grain yield and yield related traits of 18 mutant lines, parent and 2 standard varieties

Traits	Mean squares			SS, %		
	G	Y	GxY	G	Y	GxY
DH	10.16*	201.86*	3.60*	27.05	53.77	19.17
PH	132.29*	17412.40*	61.54*	6.63	87.21	6.16
SP	1.05*	8.36*	0.59*	59.70	28.24	12.06
SL	6.33*	29.93*	0.64ns	74.11	3.28	22.61
AL	5.09*	2.25*	0.78ns	77.11	7.09	15.80
PL	19.30*	17.75*	1.98*	49.00	37.33	13.67
SNS	41.75*	318.10*	5.82*	37.08	52.84	10.08
GNS	27.74*	395.40*	3.77*	24.25	72.51	3.29
GWS	0.11*	3.20*	0.01*	34.23	27.14	38.63
TGW	13.07*	636.60*	8.86*	13.83	67.40	18.77
GY	2.55*	19.09*	1.02*	39.21	29.38	31.40

* significant at 0.5% level of probability, ns- non significant; G – genotype, Y – year, GxY genotype by year interaction; DH - days to heading, PH - plant height , SP- number of spikes per a plant, SL - spike length, AL - awn length, PL - peduncle length, SNS - spikelet number per a spike, GNS - grain number per a spike, GWS - grain weight per a spike, TGW - 1000 grains weight , GY - grain yield

Table 2. Means of days to heading - DH, plant height - PH (cm), number of spikes per plant - SP, spike length - SL (cm), awn length - AL (cm) and peduncle length - PL (cm) of mutant lines, parent and standard varieties (2014/2015 - 2016/2017)

Mutant lines	DH	PH	SP	SL	AL	PL
Obzor	194.33	85.77	1.69	8.05	10.25	16.17
Emon	193.08	88.83	1.68	7.68	10.88	19.00
Kuber	**192.33**	**85.56**	**1.69**	**9.69**	**9.46**	**18.75**
16/1-1	194.17	86.54	1.86	10.63	10.21	19.29
16/1-3	193.83	90.56	2.26	9.86	9.92	18.17
16/1-4	193.67	86.52	1.67	9.01	8.42	18.08
16/1-8	193.33	85.88	2.09	9.34	9.88	20.33
16/1-10	193.58	86.38	1.72	9.68	9.50	20.42
16/1-13	193.17	82.20	1.63	9.29	9.79	17.83
16/1-14	193.33	86.57	1.63	8.98	9.67	18.38
16/1-16	192.00	86.86	1.87	9.53	9.42	20.67
16/1-21	193.00	80.58	2.16	9.44	9.58	19.04
16/2-7	193.33	85.22	2.22	9.98	9.50	19.17
16/2-9	194.00	91.88	1.77	10.16	9.88	19.25
16/2-11	193.75	88.66	1.84	9.84	9.92	18.42
16/2-19	192.33	87.18	1.54	10.36	11.08	18.08
16/2-20	190.50	77.41	2.36	9.21	9.29	17.71
16/2-23	193.00	86.93	1.98	10.22	10.04	17.25
16/3-8	192.33	89.12	1.49	9.16	9.08	15.88
16/3-12	194.00	82.17	2.40	10.40	10.92	17.00
16/3-14	192.00	87.89	1.34	9.75	10.75	19.21
LSD 0.05	*1.74*	*6.9*	*0.54*	*0.48*	*1.29*	*1.09*

positively correlated with grain yield in barley. Those results show that the increase in grain yield of the mutant lines in this study was due mainly to a higher number of spikes per plant.

Genotypic variance, phenotypic variance, genotypic coefficient of variability (GCV), phenotypic coefficient of variability (PCV), broad sense heritability, genetic advance and genetic advance expressed as percentage of the mean for the traits are presented in Table 5. Higher PCV and GCV indicate the presence of greater variability for these traits which gives enough scope for their improvement by selection. The maximum phenotypic and genotypic variability were observed for number of spikes per plant and spike length. Similar findings were reported by Shtaya et al. (2015).

Heritability in broad sense is the proportion of genetic variance in phenotypic variance, expressed as percentage. The heritability estimates in the study were classified into groups such as very high (>80%), high (60-79%), moderate (40-59%) and low (<40%) according to Pramoda and Gangaprasad (2007).

Table 3. Means of spikelet number per spike - SNS, grain number per spike - GNS, grain weight per spike - GWS (g), 1000 grain weight - TGW (g), grain yield – GY (t*ha^{-1}) of mutant lines, parent and standard varieties (2014/2015 - 2016/2017)

Genotypes	SNS	GNS	GWS	TGW	GY
Obzor	31.04	29.48	1.61	47.19	5.08
Emon	32.80	31.45	1.78	45.96	6.14
Kuber	**29.05**	**28.45**	**1.45**	**46.73**	**6.56**
16/1-1	31.60	29.62	1.60	46.02	6.75
16/1-3	29.54	28.62	1.50	46.82	7.15
16/1-4	28.76	27.69	1.39	44.53	6.69
16/1-8	29.00	27.65	1.59	46.54	7.05
16/1-10	29.20	28.78	1.58	45.96	6.54
16/1-13	28.46	27.75	1.49	43.30	6.71
16/1-14	26.17	25.03	1.45	45.14	6.91
16/1-16	28.79	27.17	1.54	44.99	6.85
16/1-21	27.44	26.81	1.49	45.34	7.23
16/2-7	31.85	30.27	1.58	46.68	7.14
16/2-9	28.99	28.21	1.47	46.84	6.81
16/2-11	27.69	27.33	1.47	45.12	6.74
16/2-19	33.05	29.90	1.49	45.60	6.39
16/2-20	27.60	26.73	1.36	44.16	6.99
16/2-23	31.34	29.28	1.47	45.63	6.41
16/3-8	27.79	26.46	1.37	45.36	6.60
16/3-12	30.73	29.64	1.53	44.29	6.98
16/3-14	30.44	29.58	1.53	44.42	6.46
LSD 0.05	*1.52*	*1.19*	*0.11*	*1.71*	*0.37*

Very high estimate of heritability was observed for spike length, awn length, peduncle length, spikelet number per spike, grain number per spike and grain weight per spike. The heritability was high for days to heading and grain yield and moderate for number of spikes per plant and plant height. High heritability indicated that the characters were less influenced by the environment. High observed heritability of these traits were in agreement with the findings of Vimal and Vishwakarma (1998), Jalata et al. (2011), Al-Tabbal and Al-Fraihat (2012), Muhammad et al. (2012), Singh (2015) and Ebadi- Segherlooet al. (2016). The low heritability was recorded for 1000 grain weight indicated that direct selection for the trait will be ineffective. Genetic advance expressed as percentage of mean was the highest for the spikelet number per spike (20.85%) followed by peduncle length (16.09%), grain number per spike (14.50%) and plant height (14.10%). Days to heading (0.58%), grain weight per spike (1.13%) and 1000 grain weight (1.58%) had lowest genetic advances as percent of mean.

Table 4. Correlation coefficients between grain yield and yield related traits

Traits	PH	SP	SL	AL	PL	SNS	GNS	GWS	TGW	GY
DH	0.394	0.046	0.070	0.145	-0.030	0.182	0.249	0.378	0.402	-0.157
PH		-0.469*	0.052	0.140	0.122	0.216	0.208	0.200	0.497*	-0.206
SP			0.262	-0.063	0.037	-0.039	-0.004	-0.012	0.071	0.534*
SL				0.138	0.178	0.128	0.079	-0.286	-0.028	0.495*
AL					-0.046	0.653**	0.635**	0.566**	0.072	-0.309
PL						-0.016	0.045	0.382	0.159	0.390
SNS							0.947**	0.626**	0.321	-0.414
GNS								0.691**	0.321	-0.396
GWS									0.379	-0.334
TGW										-0.244

** significant at the 1% level. *significant at the 5% level; DH - days to heading, PH - plant height, SP- number of spikes per plant, SL - spike length, AL - awn length, PL - peduncle length, SNS - spikelet number per spike, GNS - grain number per spike, GWS - grain weight per spike, TGW - 1000 grains weight, GY - grain yield

Table 5. Estimates of variance and genetic parameters for grain yield and yield related traits of 18 mutant lines, parent and 2 standard varieties

Traits	X	σ^2g	σ^2ph	h	PCV	GCV	GA	GAM
DH	193.10	0.55	0.85	64.56	0.48	0.38	1.13	0.58
PH	86.13	5.90	11.02	53.48	3.86	2.82	12.15	14.10
SP	1.85	0.04	0.09	43.56	16.01	10.57	0.08	4.26
SL	9.54	0.47	0.53	89.90	7.62	7.22	0.98	10.24
AL	9.88	0.36	0.42	84.74	6.60	6.07	0.74	7.50
PL	18.48	1.44	1.61	89.75	6.86	6.50	2.97	16.09
SNS	29.59	2.99	3.48	86.05	6.30	5.85	6.17	20.85
GNS	28.37	2.00	2.31	86.41	5.36	4.98	4.12	14.50
GWS	1.51	0.01	0.01	93.22	6.25	6.03	0.02	1.13
TGW	45.55	0.35	1.09	32.18	2.29	1.30	0.72	1.58
GY	6.67	0.13	0.21	59.97	6.90	5.35	0.26	3.93

X – grand mean, σ^2g - genotypic variance, σ^2ph - phenotypic variance, h (%)- broad sense heritability, PCV- phenotypic coefficient of variation, GCV - genotypic coefficient of variation, GA - genetic advance, GA (%) - genetic advance as percent of mean; DH - days to heading, PH - plant height, SP- number of spikes per plant, SL - spike length, AL - awn length, PL - peduncle length, SNS - spikelet number per spike, GNS - grain number per spike, GWS - grain weight per spike, TGW - 1000 grains weight, GY - grain yield

Since high heritability does not always indicate a high genetic gain, heritability with genetic advance considered together should be used in predicting the effect of selecting superior genotypes (Ali et al., 2002). In this study spikelet and grain number per spike showed high heritability coupled with high genetic advance. Vimal and Vishwakarma (1998) observed high heritability along with high genetic advance for length of spike, spikelets per spike and grain yield per plant in barley. Traits having high heritability combined with high genetic advance could result in a better genetic gain through selection since the variation that prevails in such a trait is due to additive gene action (Panse, 1957). Grain weight per spike showed higher heritability with low genetic advance. Similar results were reported by Muhammad et al. (2012). The high heritability coupled with low genetic advance indicates the non- additive gene effects for control, suggesting that the selection based on the phenotypic performance of this trait would not be effective (Panse, 1957).

The result of the present study showed that after seed treatment with sodium azide useful barley mutants with changes in important agronomic traits such as grain yield and yield related traits were selected. Sodium azide induced mutants with improved yield related traits were also reported by Kivi (1981) and Ulrich and Hodgdon (1985) in barley, Rachovska (1998) and Srivastava et al. (2011) in wheat, Mensah and Obadoni (2007) in groundnut, Samiullah et al. (2004) in mungbean, Khan et al. (2006) in lentil.

CONCLUSIONS

Highly significant differences among mutant lines for days to heading, plant height, number of spikes per plant, awn length, peduncle length, spikelet number per spike, grain number per spike, grain weight per spike, 1000 grain weight and grain yield were found. Mutant lines 16/1-3, 16/1-8, 16/1-21, 16/2-7, 16/2-20 and 16/3-12 had a significantly higher grain yield compared to the parent variety Kuber and standard varieties Obzor and Emon.

The improvement of grain yield in the studied set of mutant lines was associated mainly with the increase in the number of spikes per plant.

Spikelet and grain number per spike showed high heritability coupled with high genetic advance, hence these traits could be improved more easily by selection based on phenotypic performance.

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