# **Evaluation of advanced mutant lines of winter barley**

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## Abstract

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The aim of this study was to evaluate grain yield and some yield related traits of sodium azide-induced mutant lines from the winter barley varieties Zagorets, Asparuh and Imeon. Ten mutant lines from variety Zagorets, 10 mutant lines from variety Asparuh and 9 mutant lines from variety Imeon along with the 3 parent varieties were evaluated in Complete Block Design with four replications. The study was conducted in 3 years (from 2014/2015 to 2016/2017) in the experimental field of the Institute of Agriculture – Karnobat, South-eastern Bulgaria. Significant differences among the mutant lines derived from the same parental variety and also among themselves for all of the studied traits were found. Grain yield of studied mutant lines had a positive correlation with peduncle length and the grain weight per spike. The mutant lines 17/2-1, 17/1-15, 15/1-5 and 15/1-17 exhibited significantly higher and stable grain yield than the parental varieties and therefore can be selected for further evaluation for variety release and for using in the hybridization program.

Keywords: grain yield; yield related traits; sodium azide; mutants

## Introduction

Mutation induction is used for increasing variability for agronomically important traits and for obtaining genotypes more adapted to particular environmental conditions. The induced mutations have played a significant role in plant breeding in the development of crop varieties resulting tremendous economic impact on agriculture and food production that is currently valued in billions of dollars and millions of cultivated hectares (MBA, 2013).

Numerous studies have shown positive results of the applying of mutagen treatments in barley for generating genotypes with desirable traits (Maluszynski & Szarejko, 2003; Lundqvist, 2014; Ohnoutkova, 2019). Currently, in Bulgaria, there are four commercial barley varieties obtained by the Institute of Agriculture – Karnobat through mutation induction (Vulchev & Dyulgerova, 2011; Dyulgerova & Vulchev, 2012; Dyulgerova et al., 2017).

Most of the traits of interest to plant breeders were quantitative traits such as yield and yield related traits. Mutagenic induced variability in important quantitative traits like vegetation period duration, spike length, number of grains per spike, grain weight per spike, grain yield, chemical composition and physical characteristics of grain was reported in barley (Rybinski & Szot, 2006; Deniz, 2007; Gómez-Pando et al.,2009; Gómez et al., 2017)

The frequency of mutations for quantitative traits depends on the genotype of the parent variety (Siddiqui & Singh, 2010; Ambavane et al., 2015). Moreover, it was found that different polygenic traits differ in their response to the mutagenic treatments (Sharma, 1995; Khan & Wani, 2006).

Mutations can be induced by treatment with physical mutagens, such as ionizing radiation, or chemical mutagens like ethylnitroso-urea, methylnitroso-urea, ethylmethane-sulphonate (EMS) and sodium azide (SA) (Mba, 2013). Sodium azide has been used in various crops to improve their yield, quality traits and resistance against biotic and abiotic stresses (Khan et al., 2009). This mutagen is widely utilized in barley due to its ease of use and potential for producing a high frequency of mutations. The aim of this study was to evaluate grain yield and some yield related traits of sodium azide induced mutant lines from winter barley varieties Zagorets, Asparuh and Imeon

## **Material and Methods**

The study was conducted in 3 consecutive years from 2014/2015 to 2016/2017 at the Institute of Agriculture-Karnobat, South-Eastern Bulgaria. For mutation induction the pre-soaked in water for 16-hour seeds were treated with 2 mM sodium azide for 2 hours, prepared in a buffer solution (pH = 3) at room temperature and washed for 6 hours after treatment. The M1 plants grown in the field were harvested in bulk. In M2 generation one spike per selected plant was harvested and the seed of each M2 spike was sown in the field as spike to row progeny for M3 generation. The parent variety was planted in every 10 rows as a check. The mutants were developed through selection for higher yield than the parental material by applying selection pressure from M2 to M6. Finally, 10 mutant lines from variety Zagorets, 10 mutant lines from variety Asparuh and 9 mutant lines from variety Imeon along with the 3 parent varieties were evaluated in yield trial in Complete Block Design with four replications on plots of 10 m<sup>2</sup> in the present investigation. Parent varieties Zagorets, Asparuh and Imeon are winter high-yielding malting 2-rowed barley varieties, developed at the Institute of Agriculture - Karnobat (Valchev & Gocheva, 2010; Valchev & Gocheva 2012a; Valchev & Gocheva 2012b).

The studied traits included spike length (cm), awn length (cm), peduncle length (cm), spikelet number per spike, grain number per spike, grain weight per spike (g), 1000 grain

weight (g), grain yield (kg/ha). The data were recorded on a plant basis by randomly selecting 10 plants from each plot. Grain yield and 1000 grain weight were estimated on a plot basis.

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 (SPSS Inc., 2007).

### **Results and Discussion**

The analysis of variance revealed significant differences between the genotypes for all studied traits (Table 1). The effect of the year was non-significant only for the traits awn and peduncle length. Genotype by year interaction was significant for all the traits excepted awn and peduncle length in mutant lines from Asparuh and Imeon and non-significant for the spike, awn and peduncle length in mutant lines from Zagorets. Significant genotype by year interaction for grain yield indicates that the evaluated mutant lines had the differential response to the different conditions of the years of testing. Significant genotype by year interaction observed in the present study indicates that the mutant lines evaluated do not show consistent performance across test environments. This interaction complicates the selection of superior genotypes by minimizing associations between phenotypes and genotypes (Voltas et al., 2002).

Mean of studied traits of the mutant lines from Zagorets and parent are presented in Table 2. The spike length varied from 9.04 to 10.54 cm. Mutant line 18/2-5 had significantly longer spike compared to the parent. The length of awns ranged from 10.05 cm to 12.96 cm among the studied lines.

Table 1. Me Imeon	an squares for gr	ain yield and yiel	d related	traits of mu	tant lines fron	1 varieties	Zagorets, A	sparuh and
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Traits		Zagorets			Asparuh		Imeon			
	G	Y	GxY	G	Y	GxY	G	Y	GxY	
SL	2.70*	21.87*	0.46 <sup>ns</sup>	6.22*	12.45*	1.30*	3.58*	4.44*	1.44*	
AL	6.81*	0.04 <sup>ns</sup>	1.02 <sup>ns</sup>	7.57*	0.04 <sup>ns</sup>	1.66 <sup>ns</sup>	16.79*	0.20 <sup>ns</sup>	0.61 <sup>ns</sup>	
PL	659.89*	23.23 <sup>ns</sup>	91.31 <sup>ns</sup>	49.90*	15.51 <sup>ns</sup>	4.59 <sup>ns</sup>	27.54*	11.57 <sup>ns</sup>	2.82 <sup>ns</sup>	
SNS	20.03*	140.07*	10.11*	53.56*	169.93*	5.20*	31.50*	36.70*	15.74*	
GNS	22.29*	270.74*	5.95*	50.74*	176.94*	7.54*	37.32*	29.41*	16.58*	
GWS	0.09*	2.03*	0.02*	0.12*	1.37*	0.04*	0.14*	0.53*	0.14*	
TGW	44.58*	23.88*	0.94*	39.95*	28.93*	0.21*	45.00*	19.47*	1.01*	
GY	1.92*	22.44*	1.49*	0.89*	16.21*	1.15*	4.47*	5.97*	0.74*	

\*Significant at 5% level of probability; nsnonsignificant; G – genotype; Y – year; GxY genotype by year interaction; SL – spike length; AL – awn

length;PL-peduncle length; SNS – spikelet number per a spike; GNS – grain number per spike; GWS – grain weight per spike; TGW – 1000-grains weight; GY -grain yield

Peduncle length of the mutant lines varied from 15.00 cm (18/2-2) to 20.83 cm (18/3-2), while that for the parent line was 22.79 cm. Line 18/2-3 had a significantly higher spike-let number per spike than Zagorets. The higher number of grains per spike was found in the mutant lines 18/2-3, 18/2-5 and 18/2-6.Two lines (18/2-2 and 18/3-7) had lower grain weight per spike and rest mutant lines did not differ significantly from the parent. The value of 1000 grain weight of the lines 18/1-7 and 18/2-5 was higher compared to Zagorets. The mean grain yield of mutant lines from variety Zagorets varied from 5.62 t/ha to 6.9 1t/ha. The line 18/2-5 had the highest grain yield but *not significantly different than those of the* parent.

The spike length in mutants from Asparuh ranged from 8.53 cm (17/2-12) to 10.71 cm (17/2-14) (Table 3). The line 17/2-14 had longer awns than the parent. The longer peduncle was recorded in mutants 17/1-15 and 17/2-1. The higher number of spikelets and grains per spike was found in 17/1-15 and 17/2-14, whereas a lower number of spikelets and grains per spike were recorded in 17/2-3. The mutant lines 17/2-, 17/2-14and 17/2-17 showed higher grain weight per spike compared to the parent. The 1000 grain weight of the lines from Asparuh varied from 43.65g (17/2-14) to 49.47g (17/3-2). The grain yield of the 17/2-1 for the 3 year period was significantly higher and those of 17/2-13 and 17/2-14 significantly lower than grain yield of parent variety.

Table 2.	Means for	grain y	yield and	yield rela	ted traits o	f mutant lines	and parent	Zagorets (	(2014/2015 –	2016/2017)
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Mutant lines	SL	AL	PL	SNS	GNS	GWS	TGW	GY
Zagorets	9.21	11.75	22.79	27.42	26.00	1.49	46.43	6.65
18/14	9.13 <sup>ns</sup>	11.63 <sup>ns</sup>	15.79	28.00 <sup>ns</sup>	26.17 <sup>ns</sup>	1.44 <sup>ns</sup>	46.45 <sup>ns</sup>	5.62
18/15	9.38 <sup>ns</sup>	12.96 <sup>ns</sup>	18.46	29.83 <sup>ns</sup>	28.25 <sup>ns</sup>	1.49 <sup>ns</sup>	44.50	5.68
18/16	9.54 <sup>ns</sup>	11.00 <sup>ns</sup>	17.88	28.33 <sup>ns</sup>	27.00 <sup>ns</sup>	1.54 <sup>ns</sup>	45.53 <sup>ns</sup>	5.88
18/17	9.58 <sup>ns</sup>	12.33 <sup>ns</sup>	16.42	26.83 <sup>ns</sup>	25.50 <sup>ns</sup>	1.51 <sup>ns</sup>	48.73+	6.05
18/22	9.54 <sup>ns</sup>	10.92 <sup>ns</sup>	15.00	29.25 <sup>ns</sup>	26.83 <sup>ns</sup>	1.35	46.55 <sup>ns</sup>	6.06
18/23	9.7 <sup>ns</sup>	11.33 <sup>ns</sup>	18.29	30.92+	29.25+	1.52 <sup>ns</sup>	46.51 <sup>ns</sup>	6.13
18/25	10.54+	12.42 <sup>ns</sup>	18.00	29.83 <sup>ns</sup>	29.00+	1.59 <sup>ns</sup>	48.67+	6.91 <sup>ns</sup>
18/26	10.04 <sup>ns</sup>	11.08 <sup>ns</sup>	15.88	29.17 <sup>ns</sup>	28.67+	1.59 <sup>ns</sup>	46.56 <sup>ns</sup>	6.34 <sup>ns</sup>
18/32	10.25+	10.50	20.83 <sup>ns</sup>	28.92 <sup>ns</sup>	26.75 <sup>ns</sup>	1.42 <sup>ns</sup>	46.60 <sup>ns</sup>	6.00
18/37	9.04 <sup>ns</sup>	11.08 <sup>ns</sup>	16.17	27.00 <sup>ns</sup>	25.75 <sup>ns</sup>	1.34	41.65	5.76
LSD 0.05	0.93	1.24	2.43	2.99	2.48	0.11	1.26	0.36
CV,%	4.92	6.53	13.19	4.51	5.01	5.74	4.29	6.56

 $^+$ Significantly or <sup>ns</sup> not significantly different from parent variety at the 5% level of probability; SL – spike length, cm; AL – awn length,cm; PL-peduncle length, cm; SNS – spikelet number per spike; GNS – grain number per spike; GWS – grain weight per spike, g; TGW – 1000-grains weight, cm; GY – grain yield, t/ha

Table 3. Means f	for grain	vield and	vield related	traits of mu	itant lines and	parent As	paruh (	(2014/2015 -	- 2016/2017)
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Mutant lines	SL	AL	PL	SNS	GNS	GWS	TGW	GY
Asparuh	9.63	10.83	19.38	28.83	27.50	1.51	46.12	6.41
17/15	9.96 <sup>ns</sup>	11.71 <sup>ns</sup>	19.00 <sup>ns</sup>	30.00 <sup>ns</sup>	28.50 <sup>ns</sup>	1.54 <sup>ns</sup>	44.17	6.37 <sup>ns</sup>
17/18	9.46 <sup>ns</sup>	10.67 <sup>ns</sup>	19.33 <sup>ns</sup>	28.75 <sup>ns</sup>	26.92 <sup>ns</sup>	1.52 <sup>ns</sup>	44.72	6.34 <sup>ns</sup>
17/115	10.67 <sup>ns</sup>	11.29 <sup>ns</sup>	23.96+	31.75+	29.92+	1.57 <sup>ns</sup>	44.46 <sup>ns</sup>	6.73 <sup>ns</sup>
17/21	9.29 <sup>ns</sup>	10.54 <sup>ns</sup>	23.58+	31.25+	29.83 <sup>ns</sup>	1.65+	46.58 <sup>ns</sup>	6.86+
17/23	8.79 <sup>ns</sup>	10.54 <sup>ns</sup>	18.42 <sup>ns</sup>	25.00	24.17	1.43 <sup>ns</sup>	45.15 <sup>ns</sup>	6.59 <sup>ns</sup>
17/212	8.53	10.79 <sup>ns</sup>	22.04 <sup>ns</sup>	27.33 <sup>ns</sup>	25.83 <sup>ns</sup>	1.60+	43.45	6.11 <sup>ns</sup>
17/213	9.92 <sup>ns</sup>	11.29 <sup>ns</sup>	17.96 <sup>ns</sup>	30.33 <sup>ns</sup>	29.25 <sup>ns</sup>	1.51 <sup>ns</sup>	47.48+	5.99
17/214	10.71 <sup>ns</sup>	12.88+	19.96 <sup>ns</sup>	32.50+	31.08+	1.80+	43.65	6.01
17/217	10.25 <sup>ns</sup>	10.50 <sup>ns</sup>	19.08 <sup>ns</sup>	30.33 <sup>ns</sup>	29.75 <sup>ns</sup>	1.60+	44.55	6.42 <sup>ns</sup>
17/32	9.08 <sup>ns</sup>	9.88 <sup>ns</sup>	20.83 <sup>ns</sup>	29.4 ns	27.33 <sup>ns</sup>	1.47 <sup>ns</sup>	49.47+	6.50 <sup>ns</sup>
LSD 0.05	1.10	1.82	3.18	2.35	2.37	0.09	1.27	0.37
CV,%	7.45	7.23	10.03	7.14	7.29	6.42	4.02	4.36

<sup>+</sup>Significantly or <sup>ns</sup> not significantly different from parent variety at the 5% level of probability; SL – spike length, cm; AL – awn length, cm; PL – peduncle length, cm; SNS – spikelet number per spike; GNS – grain number per spike; GWS – grain weight per spike, g; TGW – 1000-grains weight, cm; GY – grain yield, t/ha

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Mutant lines	SL	AL	PL	SNS	GNS	GWS	TGW	GY
Imeon	10.29	9.92	17.88	31.92	30.83	1.58	44.69	5.96
15/13	10.25 <sup>ns</sup>	13.08+	15.38	31.33 <sup>ns</sup>	28.92 <sup>ns</sup>	1.67 <sup>ns</sup>	44.39 <sup>ns</sup>	6.83+
15/18	9.25 <sup>ns</sup>	11.08 <sup>ns</sup>	16.75 <sup>ns</sup>	27.17	25.50	1.35	47.25+	5.32
15/19	11.17 <sup>ns</sup>	11.04 <sup>ns</sup>	16.42 <sup>ns</sup>	31.92 <sup>ns</sup>	30.42 <sup>ns</sup>	1.56 <sup>ns</sup>	49.38+	5.09
15/112	9.63 <sup>ns</sup>	11.29 <sup>ns</sup>	17.08 <sup>ns</sup>	29.67 <sup>ns</sup>	28.25 <sup>ns</sup>	1.47 <sup>ns</sup>	47.64+	6.11 <sup>ns</sup>
15/117	10.46 <sup>ns</sup>	11.63+	17.33 <sup>ns</sup>	30.25 <sup>ns</sup>	28.83 <sup>ns</sup>	1.67 <sup>ns</sup>	47.36+	6.90+
15/23	10.13 <sup>ns</sup>	9.79 <sup>ns</sup>	20.29+	29.50 <sup>ns</sup>	29.75 <sup>ns</sup>	1.48 <sup>ns</sup>	45.66 <sup>ns</sup>	6.24 <sup>ns</sup>
15/32	10.21 <sup>ns</sup>	11.96+	19.38 <sup>ns</sup>	31.92 <sup>ns</sup>	29.75 <sup>ns</sup>	1.64 <sup>ns</sup>	46.80+	6.56+
15/36	9.79 <sup>ns</sup>	11.83+	18.13 <sup>ns</sup>	30.00 <sup>ns</sup>	28.50 <sup>ns</sup>	1.52 <sup>ns</sup>	47.53+	5.51
15/37	9.54 <sup>ns</sup>	10.33 <sup>ns</sup>	19.29 <sup>ns</sup>	28.33	25.92	1.42	48.38+	6.08 <sup>ns</sup>
LSD 0.05	1.15	1.63	2.34	3.31	3.27	0.15	1.23	0.38
CV, %	5.43	10.65	8.51	5.37	6.15	7.03	4.13	10.12

Table 4. Means for grain yield and yield related traits of mutant lines and parent Imeon (2014/2015 – 2016/2017)

 $^+$ Significantly or  $^{ns}$  not significantly different from parent variety at the 5% level of probability; SL – spike length, cm; AL – awn length, cm; PL – peduncle length, cm; SNS – spikelet number per spike; GNS – grain number per spike; GWS – grain weight per spike, g; TGW – 1000-grains weight, cm; GY – grain yield, t/ha

Spike length among mutants from variety Imeon ranged from 9.25 cm to 11.17 cm (Table 4). There were no significant differences between parent and mutant lines in spike length. Mutants (15/1-3,15/3-2 and 15/3-6) with longer awns were found. The peduncle length varied from 15.38 cm (15/1-3) to 20.29 cm (15/2-3). Mutant lines with a higher number of spikes and grains per spike and higher grain weight per spike compared to Imeon were not found. A significantly lower number of spikes, grains and grain weight per spike were recorded in 15/1-8 and 15/3-7. Most of the mutant lines (15/1-8, 15/1-9, 15/1-12, 15/1-17, 15/3-2, 15/3-6 and 15/3-7) had significantly higher 1000 grain weight than Imeon. The mean grain yield of 15/1-3 (6.83 t/ha), 15/1-17(6.90 t/ha) and 15/3-2 (6.56 t/ha) was significantly higher compared to the parent (5.96 t/ha).

Significant differences among the mutant lines for all of the studied traits indicating that the mutagenic treatment with sodium azide were effective in inducing mutations in these polygenic traits. Many of the mutant lines derived from the same parental variety showed significant differences from the parent and also among themselves. Mutations of quantitative traits after mutagenic treatment with sodium azide were reported in previous studies (Khan et al., 2004; Khan et al., 2006; Samiullah et al., 2004; Mensah & Obadoni, 2007).

According Mustatea et al. (2009) plotting coefficient of variation (CV) against average yield is useful in identifying varieties with high and stable yield. Figure 1 shows the yield of grain (t/ha) and the coefficient of variation (CV, %) of the grain yield for the 3 years of study. From parent varieties, Asparuh had the higher grain yield and lower CV than the average of the studied genotypes. Variety Imeon showed lower grain yield and CV and Asparuh had high but unstable

grain yield. A good combination of high grain yield and yield stability in the studied period was found in 17/2-1, 17/1-15, 15-1-5 and 15/1-17 which were superior compared to 3 parent varieties. Those mutant lines can be selected for further evaluation for variety release and for use in a future hybridization program.

The correlations between the studied traits of mutant lines are presented in Table 5. Spike length had a positive correlation with spikelet (r = 0.757) and grain number per spike (r = 0.778) and with grain weight per spike (r = 0.527). Budakli Carpici & Celik (2012) Abd El-Mohsen (2012), Gocheva



Fig. 1. Mean grain yield (2014/2015 – 2016/2017) and coefficient of variation in mutant lines and parental varieties Zagorets, Asparuh and Imeon

Traits	AL	PL	SNS	GNS	GWS	TGW	GY
SL	0.229	0.078	0.757**	0.778**	0.527**	0.175	0.067
AL		0.259	0.205	0.134	0.386*	0.056	0.038
PL			0.128	0.132	0.244	0.141	0.408*
SNS				0.947**	0.656**	0.029	0.120
GNS					0.686**	0.015	0.138
GWS						0.127	0.418*
TGW							0.097

Table 5. Correlation coefficients between grain yield and yield related traits in mutant lines from varieties Zagorets, Asparuh and Imeon

\*, \*\* Significant at 5% and 1% level of probability, respectively; SL – spike length; AL – awn length; PL – peduncle length; SNS – spikelet number per a spike; GNS – grain number per spike; GWS – grain weight per spike; TGW – 1000-grains weight; GY – grain yield

(2014) also reported a positive correlation between spike length and grain number per spike in barley. Awn length had a significant correlation with grain weight per spike (r = 0.386). Significant positive correlations were found between spikelet number per spike and grain number per spike (r = 0.947) and spikelet number per spike and grain weight per spike (r =0.656). Grain number per spike showed a significant positive relationship with grain weight per spike (r = 0.686). Grain yield had a significant and positive correlation with the peduncle length (r = 0.408) and the grain weight per spike (r =0.418). Association between peduncle length and grain yield in barley was reported by Jouyban et al. (2015), Lodhi et al. (2015) and Singh et al. (2015). A positive correlation between the grain weight per spike and grain yield was founded by Markova Ruzdik et al. (2015), Dimitrova-Doneva (2016) and Gocheva et al. (2017). No correlation was found between 1000 grain weight and grain yield. Similar results were reported by Drikvand et al. (2011) and Budakli Carpici & Celik (2012) in barley. Those results showed that from the studied traits grain weight per spike was the most important trait for the improving of grain yield of the mutant lines.

### Conclusions

This study showed that the sodium azide mutation treatment was effective for generating genetic variation for grain yield and yield related traits in winter barley.Significant differences among the mutant lines derived from the same parental variety and also among themselves for all of the studied traits were found.

Grain yield of studied mutant lines was significantly and positively correlated with peduncle length and grain weight per spike.

The mutant lines 17/2-1, 17/1-15, 15/1-5 and 15/1-17 exhibited significantly higher and stable grain yield than the

parental varieties and thereforecan be selected for further evaluation for variety release and for using in the hybridization program.

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