Evaluation of hulless mutants of winter barley

Boryana DYULGEROVA (✉)
Nikolay DYULGEROV

Summary

The aim of the present study was to evaluate the morphological and agronomic traits of seven hulless mutants of winter barley variety 'Ahil'. The study was conducted in two growing seasons at the experimental field of the Institute of Agriculture - Karnobat, south-eastern Bulgaria. The studied traits included: grain yield, number of spikes per m^2, winter hardiness, plant height, lodging, days to heading, spike length, awn length, peduncle length, spikelet number per a spike, grain number per a spike, grain weight per spike, 1000 grain weight, hectolitre weight, grain length, grain width, grain length to width ratio and protein content. The results revealed a high level of variations for studied morphological and agronomic traits among hulless mutants. Mutant lines had from 20% to 42% lower grain yield compared to the parent. The number of spikes per m^2 and winter hardiness were highly correlated with grain yield so those traits could be used in the selection of high-yielding varieties. Hulless mutants had higher grain protein content than the parent and standard varieties. Studied mutants could served as a breeding material for further improvement of hulless barley.

Key words

barley, hulless mutants, yield, yield-raleted traits

Institute of Agriculture – Karnobat, 8400 Karnobat, Bulgaria

✉ Corresponding author: bdyulgerova@abv.bg

Received: January 15, 2020 | Accepted: May 15, 2020

Introduction

The grain of hulless or naked barley compared with hulled or covered barley has many advantages when it is used for human consumption or animal feed. Increased interest in using hulless barley for food is connected with a high content of β-glucans, vitamins, minerals and proteins in its grain (Wood, 2004; Ragaee et al., 2006). Hulless barley can be used in various food products with minimal processing and with the intact bran layer to get the full benefit of the whole grain (Liu, 2007). Incorporation of naked barley in the human diet reduces the risk of coronary heart disease and certain cancers, and improve prevention and management of type 2 diabetes (Shaveta and Kaur, 2019). Naked feed barley has higher digestibility, protein and energy, and lower fiber than covered barley (Bleidere and Gaile, 2012).

The hulless grain trait is controlled by a single recessive gene *nud*, located on the long arm of chromosome 7H (Kikuchi et al., 2003). Hulless trait has been reported to be associated with reduced plant density, higher test weight, lower plant height, seed weight and grain yield (Choo et al., 2001). Contrary, Barabaschi et al. (2012) found that the differences in grain yield between hulless and hulled lines were due only to hull weight and they did not find any pleiotropic effect of *nud* gene on other traits.

The production of hulless barley mutants has been reported previously (Ehrenberg et al., 1961; Takahashi et al., 1961; Romero Loli et al., 1996). Mutants have been widely used in barley genetic studies and crop improvement. The main advantage of mutation breeding is the ability to improve one or two characteristics without altering the rest of the genotype of a well-adapted variety.

Sodium azide (NaN_3) is a powerful chemical mutagen in crop plants. The high frequency of mutations and the low frequency of chromosome aberrations make this chemical very useful for practical barley breeding (Gruszka et al., 2012)

Sodium azide has been routinely used in the barley breeding program of the Institute of Agriculture – Karnobat, Bulgaria as a mutation agent and as the result several advanced mutant lines and two varieties 'IZ Bori' and 'Bojin' have been developed.

Although barley is one of the most important cereal crops in Bulgaria the production of naked barley is insignificant since there are no high-yielding and well adapted varieties to local conditions.

The aim of the present study was to evaluate the morphological and agronomic traits of sodium azide-induced hulless mutants of winter barley variety 'Ahil'.

Materials and Methods

Seven hulless mutants obtained from winter barley variety 'Ahil' were used in this study. Parent variety and national standard varieties 'Obzor' and 'Emon' were used as controls. Variety 'Ahil' is a high-yielding malting winter variety developed at the Institute of Agriculture – Karnobat, Bulgaria.

Seeds of variety 'Ahil' pre-soaked in water for 16 hours were treated with 2 mM sodium azide for two hours, prepared in a buffer solution (pH = 3) and M1 generation was grown in growing season 2010 - 2011. The same procedure was repeated in the next growing season -2011 - 2012. Hulless mutants were selected in M2 generation - M900, M911, M917 and M983 in 2011 - 2012 and M2927, M3469 and M3579 in 2012 - 2013. Mutant lines M911 and M917 visually differ from the parent not only by the naked grain trait but they also have a six-rowed spike whereas the parent and rest of the mutant lines are two-rowed.

The field study was conducted in two growing seasons (2015 / 2016 and 2016 / 2017) at the experimental field of the Institute of Agriculture - Karnobat, south-eastern Bulgaria (42°39' N, 26°59' E). The minimal and maximal monthly temperatures and the monthly sums of precipitation during the growing season in 2015 – 2016 and 2016 – 2017 for Karnobat, Bulgaria are presented in Figure 1 and Figure 2. In both seasons the lowest temperature was measured in January –11.4°C in 2015-16 and -12.0°C in 2016-17. In the first growing season (2015-16), the total sum of precipitation was 554.4 mm and in the second growing season (2016-17) 363.2 mm. The field experiment was set up on leached chernozem soil under rainfed conditions. The design was a randomized complete block design with four replications on plots of 10 m^2. Field management followed local practices.

The studied traits included: grain yield (GY, kg/ha), number of spikes per m^2 (SM), winter hardiness (WH, scale 9 - 1, where 9 = no damage and 1 = 90 - 100% of plants dead), plant height (PH, cm), lodging (L, scale 9 - 1, where 9 = no lodging and 1 = 100% lodging), days to heading (DH), spike length (SL, cm), awn length (AL, cm), peduncle length (PL, cm), spikelet number per a spike (SNS), grain number per a spike (GNS), grain weight per spike

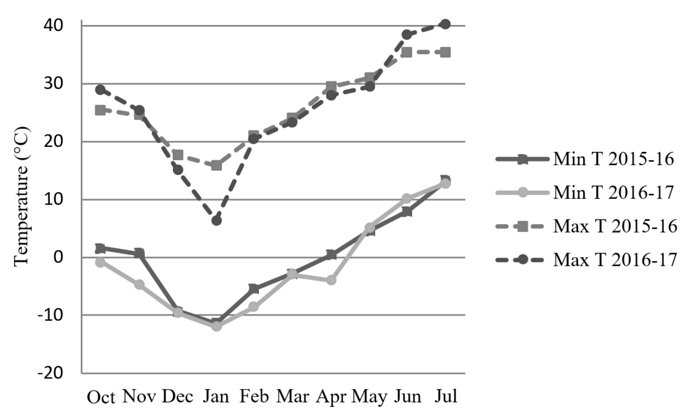

Figure 1. Minimal and maximal monthly temperatures during the growing season in 2015–16 and 2016–17 for Karnobat, Bulgaria

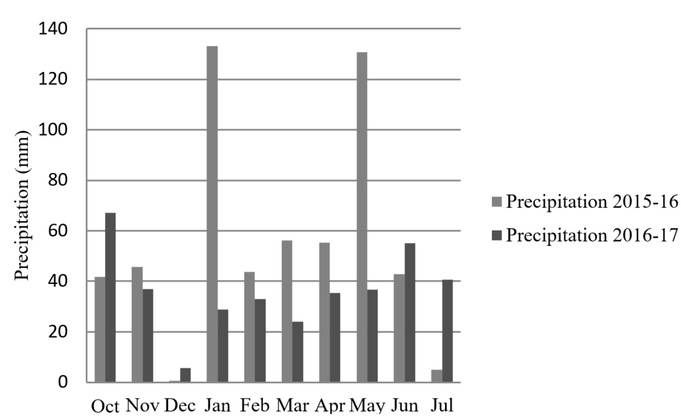

Figure 2. Monthly sums of precipitation during the growing season in 2015–16 and 2016–17 for Karnobat, Bulgaria

(GWS, g), 1000 grain weight (TGW, g), hectolitre weight (HL, kg/hl), grain length (GL, mm), grain width (GW, mm), grain length to width ratio (GL/GW), protein content (PC, %). The data was recorded on a plant basis by randomly chosen 20 plants from each plot. Grain yield, number of spikes per m^2, winter hardiness, days of heading, lodging and 1000-grain weight were estimated on a plot basis.

Data were analyzed by analysis of variance (ANOVA), correlation analysis and mean values were compared by the least significant difference (LSD) at 0.05 probability level using the SPSS19.0 software. Linear regression between grain yield and some yield-related traits was done using Microsoft Excel 2010.

Results and Discussion

Analysis of variance showed a significant effect of genotype, year and genotype x year interaction on the variation of all studied traits (Table 1). These results indicated the presence of differences among evaluated *nudum* mutants, their parent and standard varieties for investigated characteristics. Highly significant variance due to genotype x year interaction showed specific genotypic responses to environment.

Previous studies on barley mutant lines also reported significant variation of traits such as the number of tillers, plant height, weight of 1000 seeds, spike length and days to 50% heading (Obare et al., 2014).

The mean values of studied morphological and agronomic traits (2015-16 – 2016-17) are presented in Table 2. The average grain yield in mutant lines varied from 3536.3 kg/ha (M2927) to 4576.5 kg/ha (M900), while the average grain yield of the parent variety 'Ahil' was 6112.9 kg/ha. Grain yield of all *nudum* mutants was significantly lower than grain yield of parent and two standards 'Obzor' and 'Emon'. It has been reported previously that the grain yield of hulless genotypes was significantly lower than the grain yield of hulled genotypes (Choo et al., 2001; Dickin et al., 2012). Lower yield relative to that of hulled barley is the major factor limiting the acceptance of hulless barley as an alternative to hulled barley. In his study, Choo et al. (2001) found that even after grain yield of hulless lines was adjusted by adding 15% to 19% to account for hull weight loss, they still were significantly low yielding than the hulled lines. Contrary, Takahashi et al. (1961) reported that gamma-ray irradiation-induced hulless mutants yielded as much as the covered parent. In our study, the mutants showed from 20% to 42% grain yield reduction compared to the

Table 1. Mean squares of morphological and agronomic traits of nudum mutants, their parent and standard varieties

Traits	Genotype	Year	Genotype x year interaction
Grain yield	6650984.8**	22952602.5**	509876.2**
Number of spikes per 1 m^2	59260.2**	1193.5**	3536.0**
Winter hardiness	11.3**	2.1**	4.0**
Plant height	265.9**	6037.8**	120.4**
Lodging	7.8**	16.2**	7.6**
Days to heading	44.8**	756.5**	27.8**
Spike length	9.9**	21.5**	0.4**
Awn length	10.8**	13.6**	2.0**
Peduncle length	53.0**	16.8**	15.4**
Spikelet number per spike	2925.0**	3.3*	11.5**
Grain number per spike	1680.3**	37.9**	9.0**
Grain weight per spike	0.2**	3.9**	0.1**
1000-grain weight	220.8**	352.6**	13.5**
Hectolitre weight	42.9**	607.5**	2.9**
Grain length	7.0**	0.7**	1.4**
Grain width	1.6**	8.6**	1.6**
Grain length to width ratio	1.9**	12.5**	2.2**
Protein content	23.5**	0.6**	0.6**

** - significant at $P \leq 0.001$, * - significant at $P \leq 0.05$

Table 2. Mean value of studied traits of hulless mutants, their parent and standard varieties (2015-16 - 2016-17)

Genotypes	GY (kg/ha)	SM	WH (scale 9-1)	PH (cm)	L (scale 9-1)	DH	SL (cm)	AL (cm)	PL (cm)	SNS	GNS	GWS (g)	TGW (g)	HL (kg/hl)	GL (mm)	GW (mm)	GL/GW	PC (%)
Obzor	5747.4	702.5	8.9	90.3	7.0	197.0	6.9	9.50	27.7	30.5	28.9	1.23	43.10	73.2	8.2	3.4	2.5	12.4
Emon	5976.1	714.9	8.9	92.6	7.8	195.3	7.1	9.70	27.3	30.6	29.3	1.32	43.40	73.5	8.1	3.4	2.5	12.3
Ahil	6112.9	815.9	8.9	99.0	7.8	197.3	8.8	12.20	25.7	29.0	27.8	1.26	46.79	74.6	7.5	2.8	2.9	11.7
M900	4576.5	591.5	7.9	99.6	6.5	198.0	8.5	11.10	25.8	30.7	28.2	1.21	47.28	74.5	6.6	2.3	3.0	16.1
M911	4529.5	589.0	7.3	102.3	6.3	201.5	6.2	10.30	21.1	69.4	52.2	1.59	36.16	68.9	5.3	3.8	1.4	14.6
M917	4914.0	697.3	7.1	99.3	7.3	199.5	8.0	10.70	24.9	80.5	69.9	1.72	35.77	68.4	7.2	2.6	3.1	13.7
M983	4056.8	639.6	6.9	105.0	8.3	201.3	9.7	11.40	25.6	30.1	28.2	1.31	47.54	73.8	7.4	2.9	2.8	17.0
M2927	3536.3	550.4	5.5	87.3	7.8	200.5	9.1	12.70	20.0	29.7	28.5	1.41	50.93	71.9	5.8	2.7	2.4	14.3
M3469	3973.5	548.1	6.0	98.9	5.8	202.5	8.8	11.50	24.5	29.8	27.4	1.39	49.17	74.7	7.6	2.8	3.0	14.3
M3579	4068.5	598.3	7.6	91.5	5.3	197.5	8.7	12.90	27.4	29.9	26.9	1.25	48.42	74.0	7.0	2.8	2.7	15.3
LSD 0.05	*323.3*	*19.9*	*1.2*	*4.9*	*1.1*	*2.8*	*0.4*	*0.5*	*1.4*	*1.7*	*1.2*	*0.26*	*1.61*	*1.5*	*0.4*	*0.3*	*0.7*	*0.3*

GY -grain yield, SM - number of spikes per m^2, WH - winter hardiness, PH - plant height, L -lodging, DH - days to heading, 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 spike, TGW - 1000 grain weight, HL - hectolitre weight, GL - grain length, GW - grain width, GL/GW - grain length to width ratio, PC - protein content parent variety. Although the hulles lines have lower yield, it should be considered that when hulled barley is used for human food hull has to be removed, which reduces grain weight for approximately 20 - 30% (Newman and Newman, 2005).

Hulless lines had a significantly fewer number of spikes per m^2 than the parent. Murphy and Witcombe (1986) and Thomason et al. (2009) also reported that naked lines had a lower number of spikes per m2.

Despite the lack of extremely low winter temperatures during the study period the mutant lines showed lower winter hardiness compared to controls. Low winter survival of the hulless barley was noted by Dickin et al. (2012).

Nudum mutants showed variation in plant height from 87.3 to 105.0 cm. Two mutant lines (M2927 and M3579) had significantly lower and one mutant (M983) significantly higher plants in comparison to the parent. Lines M900, M911, M3469 and M3579 had lower lodging resistance than parent variety. High degree of lodging in hulless barley was observed by Dickin et al. (2012) and Sayd et al. (2018).

Most of the studied mutants (M911, M983, M2927 and M3469) had a longer period to heading than the parent. Spike length of mutant lines ranged from 6.2 to 9.7 cm. Six-rowed *nudum* mutant M911 had the shortest spike. The longest spike had M983. Hulless mutants differed from the parent in awn length (M900, M911, M917, M983, M3469 and M3579) and peduncle length (M911, M2927 and M3579). Six-rowed mutant lines had more spikelets and grains per spike, higher grain weight per spike and lower 1000-grain and hectolitre weight. There were no significant differences between the two-rowed mutant lines and the parent variety for the traits number of grains per spike and grain weight per spike. Lines M2927 and M3469 had very high 1000 grains weight 50.93 g and 49.17 g, respectively. Bleidere et al. (2013) and Sayd et al. (2018) reported that grains of hulless genotypes were characterized by higher 1000-grain weight than that of hulled barley. Although significantly higher grain volume weight has been found for hulless barley compared to hulled barley (Choo et al., 2001; Griffey et al., 2010) in our study two-rowed mutants did not differ significantly from the parent in hectolitre weight.

Grain length and grain width are important traits for determining the end-use quality value of barley grain. Heavy and plump grains are always associated with superior food and feed quality. Grain length of mutant lines M900, M911, M2927 and M3579 was lower compared to the parent. Mutant M911 had higher values for grain width. The highest grain length to width ratio was found in M917 and the lowest in M911.

The protein content of all hulless mutants was higher than that in the parent and standard varieties and varied from 13.7 to 17.0%. Mutants M983 and M900 showed the highest protein content. Many researchers have reported that hulless barley contains higher protein than hulled barley (Bhatty, 1986; Boros et al., 1996; Sayd et al., 2018).

A highly significant possitive correlation between grain yield and number of spikes per m^2 and winter hardiness was found (Table 3). The linear relationship between grain yield and studied traits showed that a unit increase in the number of spikes per m^2 and winter hardiness correspondingly increased grain yield by 9.48 kg/ha and 689.73 kg/ha, respectively (Figure 3 a, b). According to this results selection of naked genotypes with a high number of spikes per m^2 and high winter hardiness can improve grain yield. High dependence of barley grain yield on the number of productive tillers per m^2 was found by Drikvand et al. (2011), Kumar and Shekhawat (2013) and Gocheva (2014). The observed association between grain yield and winter hardiness confirms that winter survival is a critical trait for autumn-sown crops in the Bulgarian climatic conditions.

Table 3. Correlation between grain yield and morphological and agronomic traits of hulless barley mutants

Traits	Grain yield	
	r	P value
Number of spikes per m^2	0.894**	<0.001
Winter hardiness	0.904**	<0.001
Plant height	-0.044	0.903
Lodging	0.318	0.370
Days to heading	-0.831*	0.003
Spike length	-0.485	0.155
Awn length	-0.558	0.094
Peduncle length	0.545	0.103
Spikelet number per a spike	0.002	0.998
Grain number per a spike	0.036	0.922
Grain weight per spike	-0.186	0.607
1000-grain weight	-0.369	0.294
Hectolitre weight	0.087	0.810
Grain length	0.575	0.082
Grain width	0.332	0.348
Grain length to width ratio	0.047	0.898
Protein content	-0.780*	0.008

** = significant at $P \leq 0.01$, * - significant at $P \leq 0.05$

Grain yield was negatively associated with the number of days to heading and grain protein content (Table 3 and Figure 3 c, d). This relationship between yield and heading time indicates that hulless barley varieties with a long period to heading are probably not suitable for south-eastern Bulgaria because grain filling often occurs in the conditions of water deficit and high temperatures. Zaefizadeh et al. (2011) also reported a negative correlation between barley yield and days to heading in hulless barley. The negative relation between yield and grain protein content observed in our study substantiated the long-established association between these two traits in hulled barley.

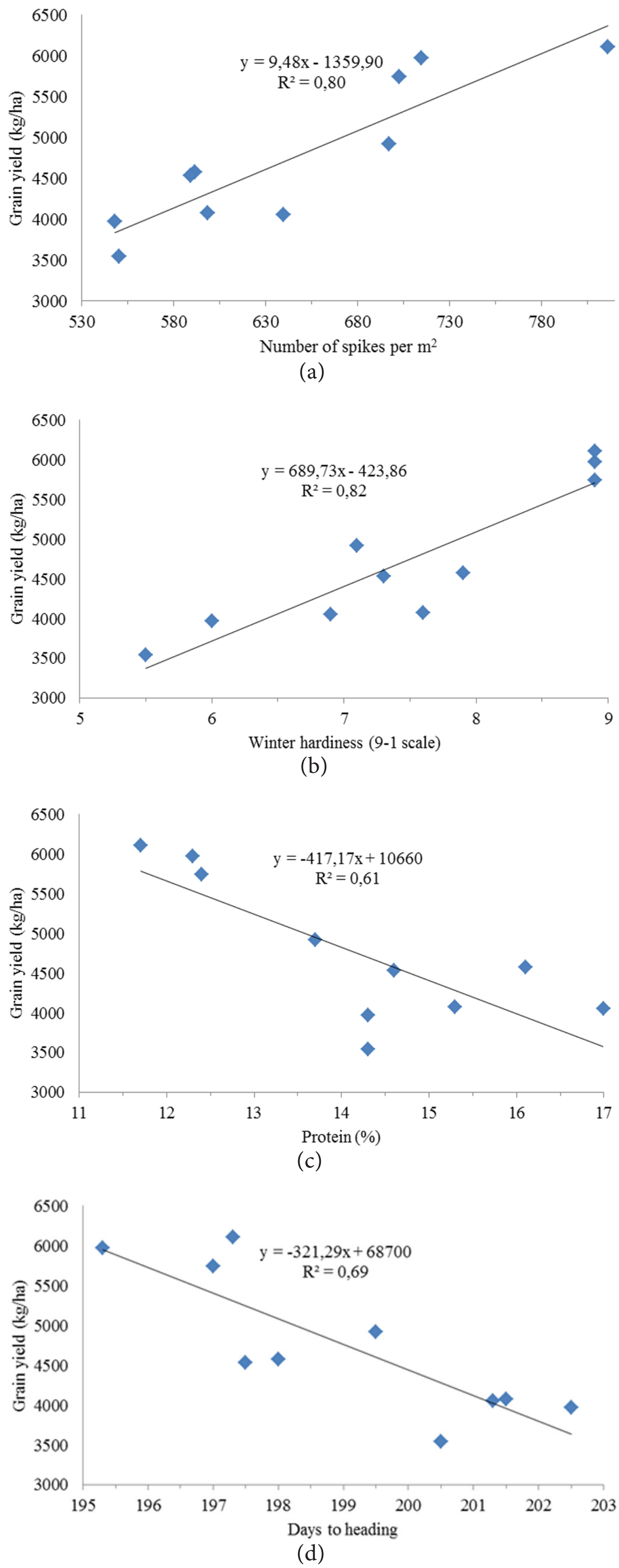

Figure 3. Linear regression between grain yield and (a) number of spikes per m², (b) winter hardiness, (c) days to heading and (d) protein content of hulless barley mutants

Conclusions

The present study revealed a high levels of variations for 18 morphological and agronomic traits among hulless mutants. The hulless mutants differed significantly from their parent variety 'Ahil' for all studied traits. Mutant lines had lower grain yield compared to the parent and standard varieties. Hulless mutants were characterized by lower number of spikes per m² and winter hardiness. These traits were highly associated with grain yield so they could be used in the selection of high-yielding genotypes for conditions of South-eastern Bulgaria. The grain protein content of mutants was higher than the parent and standard varieties. Mutant lines M983 and M900 had very high protein content. Developed mutants could served as a breeding material for further improvement of hulless barley.

Referaces

Barabaschi D., Francia E., Tondelli A., Gianinetti A., Stanca A. M., Pecchioni N. (2012). Effect of the nud gene on grain yield in barley. Czech journal of genetics and plant breeding 48(1): 10-22.

Bhatty R. S. (1986). The potential of hull-less barley – review. Cereal Chem 63: 97-103.

Bleidere M., Gaile Z. (2012). Grain quality traits important in feed barley. Proceedings of the Latvian Academy of Sciences, Section B, Versita, Vol. 66, No. 12, pp. 1-9.

Bleidere M., Zute S., Jakobsone I. (2013). Characterisation of physical and biochemical traits of hulless spring barley grain in the Latvian breeding programme. Proceedings of the Latvian Academy of Sciences, Section B, Versita, Vol. 67, No. 45, pp. 399-404.

Boros D., RekCieply B., Cyran M. (1996). A note on the composition and nutritional value of hulless barley. J Anim Feed Sci 5(4): 417-424.

Choo T. M., Ho K. M., Martin R. A. (2001). Genetic analysis of a hulless X covered cross of barley using double haploid lines. Crop Science 41:1021-1026.

Dickin E., Steele K., EdwardsJones G., Wright D. (2012). Agronomic diversity of naked barley (*Hordeum vulgare* L.): a potential resource for breeding new food barley for Europe. Euphytica 184(1): 85-99.

Drikvand R., Samiei K., Hossinpor T. (2011). Path coefficient analysis in hull-less barley under rainfed condition. Australian Journal of Basic and Applied Sciences 5(12): 277-279.

Ehrenberg, L., Gustafsson A., Lundqvist U. (1961). Viable mutants induced in barley by ionizing radiations and chemical mutagens. Hereditas 47(2): 243-282.

Gocheva M. (2014). Study of the productivity elements of spring barley using correlation and path-coefficient analysis. Türk Tarım ve Doğa Bilimleri Dergisi, 1(Özel Sayı-2): 1638-1641.

Griffey C., Brooks W., Kurantz M., Thomason W., Taylor F., Obert D., Moreau R., Flores R., Sohn M., Hicks K. (2010). Grain composition of Virginia barley and implications for use in food, feed, and biofuels production. Journal of Cereal Sci 51:41-49.

Gruszka D., Szarejko I., Maluszynski M. (2012). Sodium azide as a mutagen. In: Plant mutation breeding and biotechnology (Shu Q. Y., Foster B. F., Nakagawa H., eds.), Oxfordshire, UK, pp.159-165

Kikuchi S., Taketa S., Ichii M., Kawasaki S. (2003). Efficient fine mapping of the naked caryopsis gene (*nud*) by HEGS (High Efficiency Genome Scanning)/AFLP in barley. Theor Appl Genet 108(1): 73-78.

Kumar M., Shekhawat S. S. (2013). Research Note Correlation and path coefficient studies in barley (*Hordeum vulgare* L.) under dual purpose condition. Electronic Journal of Plant Breeding 4(4): 1313-1318.

Liu R. H. (2007). Whole Grain Phytochemicals and Health. Journal of Cereal Science 46: 207-219.

Murphy P. J., Witcombe J. R. (1986). Hulled and naked barleys from the Himalaya: 1. Evidence of multivariate differences between the two types. Theor Appl Genet 71: 730-735.

Newman C. W., Newman R. K. (2005). Hulless barley for food and feed. In: Speciality Grains for Food and Feed (Abdel-Aal E., Wood, P., eds.), St Paul: AACC Press, pp.167-202.

Obare I. J., Kinyua M. G., Kiplagat O. K. (2014). Effects of induced mutagenesis on agronomic traits in barley (*Hordeum vulgare* L). Journal of Experimental Agriculture International 4(12): 1536-1543.

Ragaee S., Abdel-Aal E. S., Noaman M. (2006). Antioxidant activity and nutrient composition of selected cereals for food use. Food Chem 98:32-38

Romero Loli M., Tafur J., Gomez L. (1996). Mutation breeding of barley in Peru. IAEA-TECDOK-869: 149-156.

Sayd R. M., Amabile R. F., Faleiro F. G., Montalvão A. P. L., Brige F. A. A., Delvico F. D. S., Sala P. I. L. (2018). Genetic parameters and agronomic characterization of hulless barley accessions under irrigation in the savanna. Rev. Bras. Cienc. Agrar., Recife, 13(3):1-8.

Shaveta H. K., Kaur S. (2019). Hulless barley: A new era of research for food purposes. Journal of Cereal Research 11(2): 114-124.

Takahashi R., Inamura H., Matsumoto T. (1961). Effects of the genes for covered and naked kernels on some agronomic characters in barley. Ber Ohara Inst Landwirtch Bio Okayama Univ 11: 385-392.

Thomason W. E., Brooks W. S., Griffey C. A., Vaughn M. E. (2009). Hulless barley seeding rate effects on grain yield and yield components. Crop science 49(1): 342-346.

Wood P. J. (2004). Relationships between solution properties of cereal beta-glucans and physiological effects-a review. Trends in Food Science and Technology, 13: 313-320.

Zaefizadeh M., Ghasemi M., Azimi J., Khayatnezhad M., Aha Dzadeh B. (2011). Correlation analysis and path analysis for yield and its components in hulless barley. Advances in Environmental Biology 5(1): 123-126.

aCS85_22