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## The variation of agronomic traits associated with breeding progress in winter barley cultivars

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### Abstract

In the 20<sup>th</sup> century, grain yield of winter barley has increased due to advances in both breeding and crop management practices. Knowledge about the association of agronomic traits with genetic gains in yield potential is necessary to improve future breeding programs. Therefore, we studied the variation in grain yield, spike length, heading date, thousand grain weight, hectolitre mass and plant height of two-rowed winter barley cultivars that were released between 1977 and 2010, in a 2-year field trial. Our results showed that most of the analyzed traits were significantly affected by the cultivar, year and cultivar-by-year interaction. The present study showed that grain yield was positively correlated with the year of release. The average rate of grain yield increase in Serbia was 46 kg ha<sup>-1</sup> per year. Further, grain yield was negatively correlated with plant height and heading date. Plant height and hectolitre mass of modern cultivars showed a significant declining trend, from the oldest to the newest cultivars. An improved understanding of the changes in agronomic traits over the last 40 years of barley breeding will help to identify targets for further breeding progress and improve the genetic potential of barley.

Key words: agronomic traits, breeding, cultivars, grain yield, *Hordeum vulgare*.

### Introduction

As a result of the rapid decline in the cropping area and the increase in human population, constant grain yield improvement of different cereal crops, including barley, represents the primary objective of breeding programs worldwide (Araus et al., 2008). Winter barley grain yield has been steadily increasing over the past century due to advances made in plant breeding and crop management practices (Peltonen-Sainio et al., 2009). Similarly, in Serbia, both areas under barley cultivation and the genetic potential of barley cultivars have undergone substantial changes over the past century.

Based on historical data, the production and breeding of barley in Serbia can be divided into three periods. Before the late 1960s, barley was cultivated in a primitive cropping system. During this period, farmers used local landraces as seed source, or developed first cultivars from local population by individual or mass selection. The second period was characterized by a shift to production of cultivars originating from Western Europe, characterized by higher grain yield and quality compared to the local population and cultivars. Starting from the late 1970s began the third period of barley cultivation and breeding, with full affirmation and domination of cultivars developed at research institutes in the former Yugoslavia (Pržulj, Momčilović, 2006).

Over the past century of barley breeding, changes in genetic potential for grain yield (Lillemo et al., 2010) were followed by significant changes in various physiological traits. For example, the development of modern high-yielding cultivars was followed by increased number of grains per unit of land area in both barley (Abeledo et al., 2008) and wheat (Foulkes et al., 2007). Under climate condition of the Pannonian plane, Mladenov et al. (2011) found that the increase in grain number per unit of land area in wheat was associated with an increase in grain number per spike and increase in spike number per square meter, while changes in thousand grain weight did not show a clear improvement trend. Further, other agronomic traits associated with grain yield, such as plant height, thousand grain weight and heading date, have similarly undergone changes during the previous period (Bertholdsson, Brantestam, 2009).

The Pannonian zone is characterized by high year-to-year climate variability, frequent water deficit and high temperature during grain filling (Pržulj et al., 2015). Furthermore, the effect of climate change is expected to additionally increase the occurrence of undesirable seasons for cereal production (Olesen et al., 2011). Knowledge about how agronomic traits associated with genetic gains in yield potential change under the climate

conditions of the Pannonian zone is crucial to the further improvement of barley breeding programs. Therefore, the aim of this study was to analyze variations in grain yield and other important agronomic traits of two-rowed winter barley cultivar released in Serbia during the last four decades.

## Materials and methods

The plant material consisted of 12 two-rowed winter barley cultivars, including 10 cultivars widely produced in Serbia during the past four decades (1977–2010) and two advanced lines developed at the Institute of Field and Vegetable Crops, Serbia (Table 1). The experimental plot size was 1.0 m wide and 5 m long,

with 0.1 m spacing between rows, and a planting density of 450 grains per m<sup>2</sup>. The trials were conducted during two growing seasons (2012–2013 and 2013–2014) at Rimski Šančevi experimental field (45°20' N 19°51' E) near Novi Sad, Serbia. A randomized complete block design with three replications was used. Each year the plots were rotated with soybean (*Glycine max* L.). Standard agricultural practices were conducted in both seasons. Nitrogen (N) fertilizer was applied in two rates: 45 kg ha<sup>-1</sup> before sowing (NPK 15:15:15, 300 kg ha<sup>-1</sup>) and 45 kg ha<sup>-1</sup> (calcium ammonium nitrate, N 27%) at tillering stage (late February or early March). Diseases and insects were controlled by spraying pesticides at the rates recommended by manufacturers.

**Table 1.** Name, year of release and pedigree of 12 winter barley cultivars developed in Serbia over the past 40 years

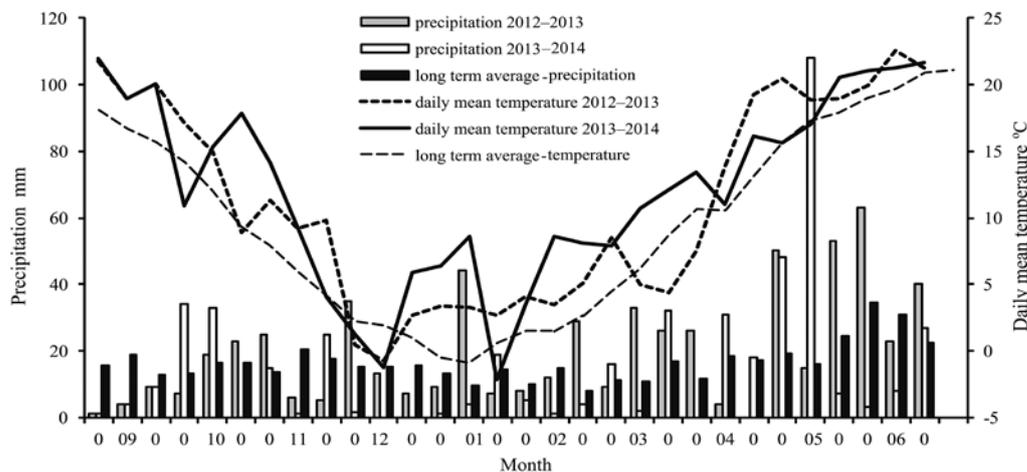
Cultivar	Year of release	Pedigree
Novosadski 183	1977	Ager/Emir
Novosadski 293	1982	FR 33/NS 185-2
Novosadski 295	1982	FR 33/NS 190
Novosadski 311	1985	NS 185-2/FR 33
Novosadski 331	1985	NS 185-2/2*FR 33
L.150/88	1988	Aksamit/NS 188//Sonja
L.147/94	1994	Nepal 81/2*TU10-76//Novosadski 293
Novosadski 519	1998	Rodnik/Corona
Novosadski 525	1999	NS Ranij-1/Novosadski 293//NS 327/3/Sladoran
Novosadski 565	2003	Sonate/Danko
Novosadski 595	2007	Sonate/Novosadski 525
NS Pinon	2010	Novosadski 525/Tiffany//Angora/Vanessa/3/Metal/Korsö

The crop was machine-harvested and grain yield (t ha<sup>-1</sup>) was adjusted to 14% moisture. Thousand grain weight (g) was determined by measuring three sets of 300 grains per plot and expressed as the weight of 1000 grains. Hectolitre mass (kg hl<sup>-1</sup>) was determined by measuring a known volume of the natural seed sample. Heading date (days) was calculated as the number of days from 1<sup>st</sup> January to the heading date. Heading date was recorded at the day when at least 50% of the spikes in a plot had emerged fully (growth stage 55) (Zadoks et al., 1974). Plant height (cm) and spike length (cm) were recorded for each plot or the mean of 10 randomly selected plants in the inner rows of each plot.

Data were processed using a two-way analysis of variance. Means were compared using Tuckey's multiple range test. Principal component analysis (PCA) was used

to determine interdependence between the traits. All analyses were performed in *STATISTICA 10*.

**Weather conditions.** Weather records (decade rainfall and average temperatures) were obtained from a meteorological station placed at the same location and reported as mean decade data together with 45 year averages for temperature and rainfall (Fig. 1). In the 2012–2013 growing season, the weather conditions in the winter period were similar to the long-term average, while in the following season the winter was mild with extreme drought. Optimal conditions during the spring 2013 and 2014, namely increased daily temperatures and abundant precipitations, favoured a vigorous plant growth. Excessive rainfall and an average temperature accompanied grain development in May of 2013 and 2014. In both growing seasons during June, grain filling



**Figure 1.** Ten-day period average temperature and precipitation for the 2012–2013 and 2013–2014 growing season and long term average (1968–2013)

of barley cultivars occurred under high daily mean temperatures over long term average, with maximum day temperatures exceeding 35°C.

## Results and discussion

Different agronomic traits (grain yield, heading date, spike length, plant height, hectolitre mass and thousand grain weight) of the 12 barley cultivars from Serbia included in this trial are shown in Table 2. Most of the analyzed traits were significantly affected by the influence of cultivar (C), year (Y) and cultivar-by-year interaction (C × Y).

Yield of different barley cultivars varied from 8030 to 10700 kg ha<sup>-1</sup>. The mean grain yield of all cultivars was 9173 kg ha<sup>-1</sup> (Table 2). In cereal breeding, grain yield represents one of the most important and complex traits, and during the development of new cultivars, the continuous grain yield improvement remains a top priority (Yan et al., 2007). The present study showed that grain yield was positively correlated with the year

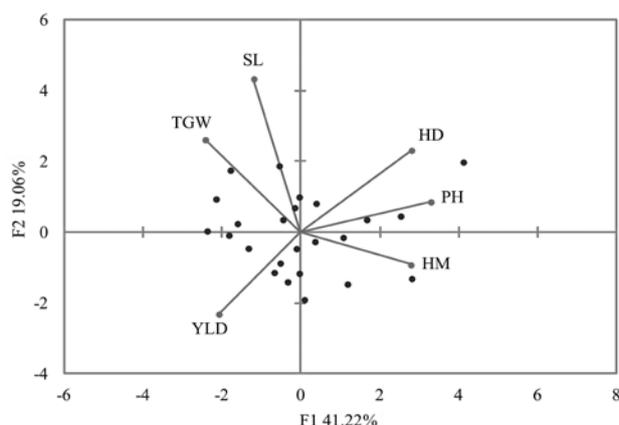
of release. The average rate of grain yield increase per year in Serbia was 46 kg ha<sup>-1</sup> (Fig. 3D). Similar results of genetic improvement per year of release in barley were previously reported by Rijk et al. (2013) for conditions of the Netherlands and Laidig et al. (2014) for Germany. Also, a positive trend of grain yield increase was found for wheat in Serbia (Mladenov et al., 2011) and other small grain crops worldwide (Araus et al., 2008).

Further, the association between grain yield and other agronomic traits is illustrated by the biplot of the principal component analysis (PCA) (Fig. 2). The first PCA explained 41.22% of total variation, while the second PCA explained 19.06%. Together, both axes accounted for 60.28% of the total variation in the data. According to the biplot, PCA1 relates predominantly to plant height and hectolitre mass, while PCA2 was mostly related to spike length. According to this biplot, strong negative association was found between grain yield and plant height, and grain yield and heading date, as indicated by the obtuse angle. Yield was additionally negatively associated with hectolitre mass and spike

**Table 2.** Mean values and analysis of variance (significance) for different agronomic traits of 12 winter barley cultivars developed in Serbia over past 40 years

Cultivar	Heading date days	Spike length cm	Plant height cm	Hectolitre mass kg hl <sup>-1</sup>	Thousand grain weight g	Grain yield kg ha <sup>-1</sup>
Novosadski 183	121 b	9.9 cd	100.0 abc	76.1 a	44.3 cd	9036 bcd
Novosadski 293	120 bc	9.5 de	100.5 ab	74.9 ab	44.7 bcd	8299 de
Novosadski 295	125 a	9.5 de	105.5 a	75.4 a	45.5 abcd	8030 e
Novosadski 311	119 bc	9.5 de	95.5 bcd	74.7 ab	44.6 bcd	8950 bcd
Novosadski 331	118 c	9.0 e	97.0 bcd	73.9 ab	43.1 d	9513 ab
L.150/88	120 bc	9.0 e	98.0 abcd	74.9 ab	44.6 bcd	8930 bcd
L.147/94	121 bc	11.0 ab	97.0 bcd	73.5 ab	45.7 abcd	8620 cde
Novosadski 519	119 bc	10.5 bc	91.0 d	73.6 ab	44.6 bcd	9371 abc
Novosadski 525	119 bc	11.5 a	93.5 bcd	74.6 ab	47.3 abc	9422 ab
Novosadski 565	121 b	10.5 bc	94.5 bcd	74.0 ab	47.7 ab	9954 a
Novosadski 595	119 bc	10.0 cd	90.5 d	73.2 ab	46.0 abcd	9878 a
NS Pinon	120 bc	9.5 de	92.5 cd	72.4 b	48.1 a	10070 a
Average	120	9.9	96.3	74.2	45.2	9173
2012–2013	119 b	9.89 a	94.5 b	73.7 b	47.6 a	9011 b
2013–2014	121 a	10.0 a	98.1 a	74.8 a	43.4 b	9335 a
Cultivar (C)	**	**	**	**	**	**
Year (Y)	**	ns	**	**	**	**
C × Y	**	**	**	ns	**	*

\*\* – significance at 0.01 probability level, \* – significance at 0.05 probability level; ns – non significant



SL – spike length, TGW – thousand grain weight, YLD – grain yield, HD – heading date, PH – plant height, HM – hectolitre mass

**Figure 2.** Principal component analysis (PCA) of trait association in two-rowed winter barley across two growing seasons

length. Generally, grain yield had a near zero or no association with thousand grain weight.

Optimal heading date is a significant trait determining cultivar adaptation to particular environments (Borràs-Gelonch et al., 2010). It was observed that the average heading date was 2 days later in 2013–2014 compared to 2012–2013. Also, a clear difference between cultivars was recorded in heading date, ranging from 118 days for ‘Novosadski 331’ released in 1998, to 125 days for ‘Novosadski 295’ released in 1982. Although heading date varied significantly between cultivars, no clear improvement trend according to the year of release was observed (data not shown). In addition, in oats, heading date was not significantly correlated with the year of release (Peltonen-Sainio et al., 2007). Despite the absence of a clear trend in heading date respective to breeding, Foulkes et al. (2009) reported that the increase in duration of stem elongation remains a significant goal in modern cereal breeding. Due to high association between the duration of stem elongation phase and final grain number, the prolongation of this phase is suggested as a potential method for further grain yield improvement (Borràs et al., 2009).

According to Figure 2, heading date was negatively associated with grain yield, indicating that barley cultivars with a long period prior to heading are not suitable for semi-arid conditions of the Pannonian plane. In such conditions, grain filling of late heading date cultivars often occurs in unfavourable environments characterized by high temperatures and water deficit. On the other hand, Pržulj and Momčilović (2006) stated that very early barley cultivars had lower yield potential and are not suitable for climate conditions in Pannonian plane. These cultivars had a short vegetation period and could not accumulate sufficient level of assimilate for grain growth during pre- and post-anthesis period (Miroslavljević et al., 2015 b). Therefore, medium early cultivars have an optimal phenological development for conditions of the Pannonian plane and are more suitable for large scale production.

As a result of positive association with grain number per spike, spike length is one of the most important yield components in breeding for increased grain number per spike and yield in six- and two-rowed barley (Madic et al., 2009). Average of spike length in this study was 9.9 cm. On average, 'Novosadski 525' and L.147/94 had the highest spike length, while the lowest was recorded in L.150/88, 'Novosadski 331', 'NS Pinon', 'Novosadski 293', 'Novosadski 295' and 'Novosadski 311'. However, our results showed that correlation coefficients between the year of cultivar release and spike length were statistically non-significant (data not shown), while the relationship between spike length and grain yield was slightly negative (Fig. 2). Therefore, barley breeders should be more focused on the improvement of spike density, to achieve further grain number per spike and grain yield increase (Pržulj, Momčilović, 2006).

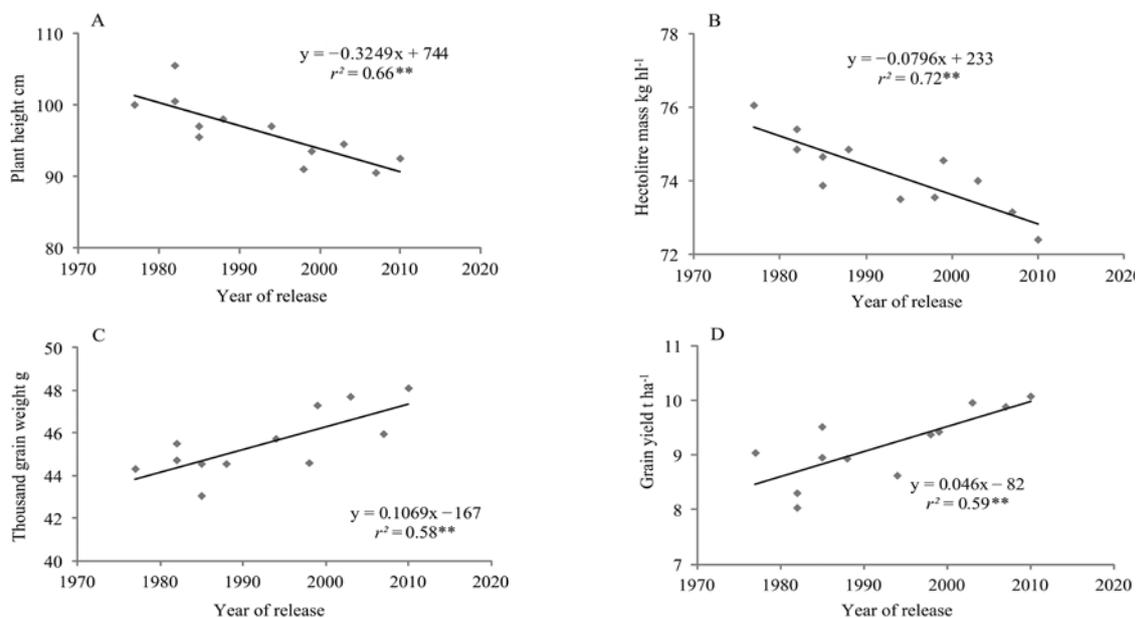
In addition to the increase in grain yield, the improvement of malting quality is a significant aim in barley breeding. Malting quality is a complex trait that consists of several components. The main objectives for malting barley breeding are the improvement of grain quality traits such as malt extract and protein content, and grain physical traits including grain formation and weight

and glume content (Matthies et al., 2014). It should be emphasized that during the process of barley breeding, grain quality is mainly tested in later generations, which additionally underlines the importance of grain weight and size in development of new malting barley cultivars.

Variation in thousand grain weight is under strong influence of genetic and non-genetic factors (Pržulj et al., 2014). Our results also showed that Y, C and C × Y significantly affected thousand grain weight. The mean thousand grain weight of different barley cultivars used in this study was 47.6 g in 2012–2013 and 43.4 g in 2013–2014. Cultivars 'NS Pinon', followed by 'Novosadski 565' and 'Novosadski 525', had the highest thousand grain weight. Further, the year of release was significantly correlated with thousand grain weight (Fig. 3C) and over the past four decades of breeding, barley thousand grain weight increased by 0.107 g year<sup>-1</sup>. Conversely, Bertholdsson and Brantestam (2009) reported a trend of linear decrease in thousand grain weight in winter barley registered in different decades.

PCA showed that grain yield had no relationship with thousand grain weight. The absence of a relationship between grain yield and thousand grain weight can be explained by the observation that in the last decades grain yield improvement was closely correlated to the increase of grain number per unit area both in barley (Bingham et al., 2012) and wheat (Sanchez-Garcia et al., 2012), and was not associated with the increase of grain weight (Zhou et al., 2014). Although we found that grain yield was not associated with thousand grain weight, the malting industry will continue to impose additional criteria regarding development of new cultivars with its strict quality requirements. Therefore, new barley cultivars should have increased grain number per unit area and should retain high grain weight and quality.

In general, plant height is an important trait for cereal production, and in this study it was affected by the year (Y), cultivar (C) and their interaction (C × Y). These results are in agreement with reports by other authors for spring and winter barley (Pržulj et al., 2014; Miroslavljević et al., 2015 a). In our study, the



Note. Determination coefficient ( $r^2$ ) and probabilities are given \*\* –  $P < 0.01$ .

**Figure 3.** Linear regression on year of release for plant height (A), hectolitre mass (B), thousand grain weight (C) and grain yield (D) for the set of 12 cultivars released from 1977 to 2010

plant height of different cultivars varied across years, ranging from 105.5 cm for cultivar released in 1982 ('Novosadski 295') to 90.5 cm for the cultivar released in 2007 ('Novosadski 595'). Plant height is positively associated with susceptibility to lodging, and high plants often had reduced grain yield and quality (Berry et al., 2007). Figure 2 shows the negative relationship between plant height and grain yield. The reduction of plant height represented an important goal during the 20<sup>th</sup> century in barley (Bertholdsson, Brantestam, 2009) and bread wheat (Diederichsen et al., 2013). Our results suggest that the decrease in plant height still remains an important goal in barley breeding programs. According to Figure 3A, plant height of modern cultivars showed a decreasing trend from the oldest to the newest, with an average decrease of 0.32 cm per year of release. It has been stated that the plant height of modern barley cultivars is within the optimum range (70–100 cm) for optimum grain yield, and barley breeders should not further decrease plant height below these values (Foulkes et al., 2009).

Although hectolitre mass has lost on significance in the past decades, it is still an important indicator of barley quality. Fox et al. (2006; 2007) found positive association between hectolitre mass and different malt and feed quality. Variation in hectolitre mass was significantly affected by C and Y, while the influence of C × Y was not significant. Among the tested cultivars, old barley cultivar 'Novosadski 183' had the highest average hectolitre mass (76.1 kg hl<sup>-1</sup>), while modern 'NS Pinon' had the lowest (72.4 kg hl<sup>-1</sup>). Although the differences between the cultivars were small, hectolitre mass showed a slight but significant decreasing trend in respect to the year of release (Fig. 3B). The average rate of hectolitre mass reduction per year of release was 0.03 kg ha<sup>-1</sup> per year.

Apart from the increase in thousand grain weight and reduction in plant height, one of the potential goals in the cereal breeding program should be to increase the above ground plant biomass (Araus et al., 2008). Modern cultivars have already achieved high values of harvest index, and further improvement of barley and wheat cannot be associated with additional harvest index increase (Foulkes et al., 2007). Therefore, Zhou et al. (2014) suggested that further improvement of grain yield potential should be achieved by increasing the biomass production, while maintaining or even increasing the harvest index.

## Conclusions

1. Barley grain yield was positively correlated with the year of release. The average rate of grain yield increase for the period 1977–2010 was 46 kg ha<sup>-1</sup> per year of release. These results indicate that increase of grain yield was the main objective of barley breeding, and will remain so in the development of new cultivars.

2. Under the conditions of the Pannonian plane, the development of new barley two-rowed cultivars was followed by the increase in thousand grain weight, and decrease in plant height and hectolitre mass. However, there was no significant correlation between heading date and spike length with the year of cultivar release. As a result of high demands of malting industry and crop management, barley breeding should be directed toward development of new cultivars characterized by decreased plant height and good grain quality.

3. A better understanding of the changes in the agronomic traits over 40 years of barley breeding helps us to identify targets for further breeding programs, with the aim of increasing the grain yield potential of modern barley cultivars.

4. Further improvement of modern barley cultivars should be achieved by integration of conventional plant breeding with modern molecular markers technology.

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## Žieminių miežių veislių agronominių savybių kaita susijusi su selekcijos pažanga

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### Santrauka

Dvidešimtajame amžiuje žieminių miežių derlius padidėjo dėl selekcijos ir augalų auginimo agrotechnikos pažangos. Žinios apie agronominių savybių ryšį su genetiniais pasiekimais, susijusiais su derliaus potencialu, yra svarbios siekiant patobulinti ateities selekcinės programas. Tuo tikslu buvo atliktas dvejų metų lauko bandymas, kurio metu tirtas derliaus, varpos ilgio, plaukėjimo datos, tūkstančio grūdų masės, saiko svorio ir augalų aukščio variavimas dvieilėse žieminių miežių veislėse, išvestose 1977–2010 m. Tyrimų rezultatai parodė, kad daugumai iš tirtų agronominių požymių didelę įtaką turėjo veislė, metai ir veislės bei metų sąveika. Taip pat nustatyta, kad grūdų derlius teigiamai koreliavo su veislės išvedimo metais. Vidutinis miežių grūdų derliaus padidėjimas Serbijoje buvo 46 kg ha<sup>-1</sup> per metus. Be to, grūdų derlius neigiamai koreliavo su augalų aukščiu ir plaukėjimo data. Šiuolaikinių veislių augalų aukštis ir saiko svoris parodė esminę mažėjimo tendenciją nuo seniausių iki naujausių veislių. Geresnis miežių agronominių savybių pokyčių per pastaruosius 40 selekcijos metų supratimas padės nustatyti ateities selekcijos pažangos tikslus ir pagerins miežių genetinį potencialą.

Reikšminiai žodžiai: agronominiai požymiai, grūdų derlius, *Hordeum vulgare*, selekcija, veislės.

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