Changes in leaf appearance and developmental phases associated with breeding progress in six-rowed barley in the Pannonian Plain

Milan Mirosavljević¹, Vojislava Momčilović*, Sanja Mikić², Ljiljana Brbaklić¹, Dragana Trkulja¹ and Ivan Abičić²

Abstract: The aim of this study was to analyze traits of leaf appearance and phenological development related to grain yield gain in winter barley cultivars released over the past 50 years. Field trials with 15 six-rowed winter barley cultivars were conducted during two growing seasons. The main leaf appearance and developmental traits were studied. The duration of the emergence-anthesis and emergence-physiological maturity phases decreased by 2.35 and 2.16 GDD yr⁻¹, respectively. The duration of the stem elongation-anthesis phase was 10% longer in modern cultivars. The results showed no clear trend of improvement in final leaf number and phyllochron. The duration of the stem elongation-anthesis phase was positively related with grain number and yield. Constant improvements in grain number and grain weight by fine manipulation of the crop developmental phases could represent an essential strategy for further increases in barley grain yield potential.

Keywords: Grain filling, Hordeum vulgare L., leaf number, phyllochron, pre-anthesis development.

INTRODUCTION

In small grain cereals, crop development represents one of the most important single factors, influencing crop performance and adaptation. Although grain yield is formed continually during the entire period from sowing to maturity, certain developmental stages are more decisive than others in determining the final grain yield (Slafer 2003). For example, in different cereal crops, stem elongation has been recognized as a crucial phase for the determination of grain number per spike and unit area (GN) (González et al. 2011), while the periods from booting to anthesis (Ugarte et al. 2007) and grain filling period (Mirosavljević et al. 2018a) are essential for grain growth and final weight. In addition, anthesis date has also been identified as an important trait for the adjustment of the crop phenology to the available resources and for cultivar adaptation to specific environmental conditions (Reynolds et al. 2009).

Different rates of genetic improvement were recorded for the main cereal crops on the Pannonian Plain (Mladenov et al. 2011, Mirosavljević et al. 2016). For barley grain yield potential in the past century (Lillemo et al. 2010), the improvement was associated with significant changes in key agronomic (Ortiz et al. 2002) and physiological traits (Abeledo et al. 2004). Generally, in different cereal crops, grain yield progress was mainly related with a higher GN (Zhou et
Changes in leaf appearance and developmental phases associated with breeding progress in six-rowed barley in the Pannonian Plain al. 2014), although some studies (Morgounov et al. 2010, Aisawi et al. 2015) identified a significant linear increase in grain weight (GW) with year of cultivar release (YCR). Also, different physiological traits such as photosynthetic activity, stomata conductance, chlorophyll level (Beche et al. 2014) and stay-green pattern (Kitonyo et al. 2017) were changed by breeding.

Modifications of the duration of different developmental phases in durum (Isidro et al. 2011) and bread wheat (Beche et al. 2018) were related to the genetic improvement of the yield potential. However, information about changes in the pre-anthesis duration and different leaf appearance traits in six-rowed winter barley for the conditions of the Pannonian environment are not available. The Pannonian Plain is one of the most vulnerable European zones, negatively affected by the influence of climate change (Olesen et al. 2012). Adverse impacts of these changes in Europe, reflected in temperature rises, precipitation decrease and increased occurrence of temperature and precipitation extremes (IPCC 2013) are already significantly affecting the crop phenology and production in Europe (Mäkinen et al. 2018). In order to mitigate the negative influence of climatic changes, crop management practices and plant breeding programs should focus more on adjusting the crop phenology to the predicted climate variations (Trnka et al. 2014).

Information about changes in the duration of specific phenological phases associated with genetic gain in grain yield could be useful to improve cereal breeding programs. Therefore, the objectives of this study were to analyze the influence of barley breeding programs of the past 50 years on the duration of the developmental phases and to evaluate the importance of each phase for grain yield determination of six-rowed winter barley on the Pannonian Plain.

MATERIAL AND METHODS

Cultivars and experimental setup

In this study, 15 six-rowed winter barley cultivars, widely grown in Serbia and the surrounding countries on the Pannonian plain, released between 1972 and 2013, were evaluated to investigate changes in the developmental pattern and leaf appearance traits. According to their year of release (Table 1A), the cultivars were grouped in four periods: period I - cultivars released prior to 1979 (Majo, Novosadski 27, Robur and Novosadski 150); period II - cultivars released between 1980 and 1989 (Kredit, Okal, Novosadski 313 and Novosadski 317); period III - cultivars released between 1990 and 1999 (Novosadski 703, Botond, Atilla and Galeb); and period IV - cultivars released after 2000 (Nonius, Rudnik and Carmina). The study was conducted in the 2015/16 and 2016/17 growing seasons on the experimental field of the Institute of Field and Vegetable Crops (lat 45° 20´ N, long 19° 51´ E), Novi Sad, Serbia, on a non-carbonate chernozem soil.

In each growing season, the cultivars were sown, arranged in a randomized complete block design with three replications. The plots were 1.0 m wide and 5 m long, with 0.1 m spacing between rows and a plant density of 350 grains per m$^2$. Prior to sowing in both growing seasons, a NPK fertilizer (containing 15% N, 15% P$_2$O$_5$ and 15% K$_2$O) was applied to avoid N, P and K deficiency (average applied rate ca. 50 kg ha$^{-1}$ of N, 60 kg ha$^{-1}$ of P, and 60 kg ha$^{-1}$ of K). In early February, additional nitrogen fertilizer (ammonium-nitrate - 33% N) was top-dressed, according to N-min analysis, at average rates of 50 kg ha$^{-1}$ of N, in both growing seasons. When necessary, weeds were periodically removed by hand. As required, the chemical control of pests (deltametrine) and diseases (tebuconazole and protiokonazol) was applied at appropriate rates in spring.

Data recording

The duration of the following developmental phases was determined: emergence-tillering (EM-TL), tillering-stem elongation (TL-SE), stem elongation-anthesis (SE-AN), emergence-anthesis (EM-AN), anthesis-physiological maturity (AN-PM) and emergence-physiological maturity (EM-PM). The dates of emergence (Z10), tillering (Z21), stem elongation (Z31), anthesis (Z65) and physiological maturity (Z89) were recorded for each plot when 50% of the plants reached the respective stage (Zadoks et al. 1974). To study the dynamics of leaf appearance, three plants were labelled in the inner rows of each sub-plot at seedling emergence. Leaf number on the main stem (FLN) was measured three times per week, by the Haun (1973) scale. Phyllochron (PHY) was calculated as the reciprocal of the slope of the relationship between the cumulative number of emerged leaves and thermal time. Daily maximum (Tmax) and minimum (Tmin) air temperatures were obtained from the meteorological station located in the experimental field. Daily mean air temperature was calculated as $T = 0.5 \times (T_{max} + T_{min})$. Duration of the phases was expressed in accumulated thermal time (GDD) between the particular stages.
The plants were harvested with a combine harvester (Wintersteiger Delta) and grain yield (GY) (t ha\(^{-1}\)) was adjusted to 14% moisture. Grain weight was determined by measuring three sets of 300 grains per plot and expressing the weight of individual grains. Grain number per unit area was calculated as the ratio between grain yield and grain weight.

### Statistical analysis

Analysis of variance (ANOVA) was performed considering environment as a random factor. Treatment means were compared by the Tukey test. Absolute and relative genetic gains were computed as a slope of the linear regression between the absolute or relative value of the trait and the year of cultivar release. Relative values were computed for each variety as percentage of the mean values of all cultivars. Principal component analysis (PCA) was performed on...
the correlation matrix between means of each variety to determine the relationships between growth traits, final grain weight and yield per hectare. All analyses were performed using R 3.0.2 (R Development Core Team).

Meteorological conditions

The weather data from the meteorological station in the experimental field indicated that the precipitation amount and distribution were similar in both growing seasons, especially during the early growth stage. In 2015/16, the total sum of precipitation from September to June was 662 mm, while the following season was characterized by a lower level (464 mm). This annual difference in precipitation was mainly observed during winter dormancy (January, February and the beginning of March) and at the end of June when the crop cycle was already completed. The 2016/17 growing season was characterized by lower temperatures in the winter period (December, January and February), and relatively higher temperatures in spring (March, April and May).

RESULTS

Main agronomic traits

The results showed differences between breeding periods for lodging, plant height, grain weight and grain yield. In addition, the analyzed traits varied notably among cultivars from the same breeding period (Table 1B). During the past 50 years, GY increased by 25%, and the minor improvement was between period III and IV (5%). The cultivars of breeding period II had a 9% better performance than those of period I, while the cultivars of period III produced an additional yield increase of 9% compared to those of period II. Moreover, modern cultivars (breeding period IV) were characterized by decreased lodging susceptibility and shorter plant height, while GW was increased compared to cultivars from the previous breeding periods.

Leaf appearance traits

The results showed that year (Y), period (P), cultivar (C) and Y × C interaction had a significant influence on the two leaf appearance traits FLN and PHY (Table 2A and 2B). The cultivars of period IV produced the lowest number of leaves per main stem (13.6) and those of period III the highest number (14.5). In 2015/16, FLN was higher (14.6) than in 2016/17 (13.5). In the different periods, PHY varied from 69.4 (period III) to 74.8 GDD leaf⁻¹ (period I), and from 70.1 (in 2016/17) to 74.5 GDD leaf⁻¹ (in 2015/16). Although FLN and PHY varied significantly between cultivars, no clear trend of trait improvement was observed based on YCR.

Duration of developmental phases

Duration of EM-TL was affected by the significant influence of Y, C and their interaction (Y × C). Although there was a clear genotypic variation in EM-TL duration, no significant difference in duration between breeding periods was observed. The duration of the EM-TL phase averaged across genotypes was longer in 2015/16 (217
GDD) than in the second growing season (199 GDD). The TL-SE phase duration was significantly affected by Y, P, C and the Y × C interaction. The longest TL-SE phase was recorded in old cultivars, in breeding period I (548 GDD), while in the modern cultivars (period IV) this phase was the shortest (420 GDD). Averaged across cultivars, duration of TL-SE was 516 and 467 GDD in 2015/16 and 2016/17, respectively. The SE-AN phase was significantly affected by all sources of variation (Y, P, C, Y × P and Y × C). Among breeding periods, the SE-A period was the longest in the modern cultivars, while the shortest duration of this period was observed in older cultivars (breeding period I). The mean duration of SE-AN of different six-rowed barley cultivars released in the Pannonian Plain in the past 50 years was 532 GDD in 2015/16 and 506 GDD in 2016/17, with an overall average of 519 GDD.

The duration of the studied developmental phases is shown in Figure 1A. Comparing modern cultivars of breeding period IV with older cultivars (breeding period I), the TL-SE phase was 23.3% shorter for modern cultivars, representing a decreased rate of -3.82 with YCR. On the other hand, the SE-AN period in modern cultivars was increased by 10% in comparison to old cultivars. The SE-AN phase increased significantly with year of cultivar release, with relative and absolute genetic gains of 1.48 GDD and 0.29% with YCR, respectively. The variation in the period duration from anthesis to physiological maturity (AN-PM) was significantly influenced by Y, P, C and Y × C. The longest grain filling period was reported in cultivars of breeding period III, while the shortest duration was observed in breeding period I. Averaged across cultivars, the AN-PM phase lasted 575 and 542 GDD in 2015/16 and 2016/17, respectively. The duration of grain filling period (AN-PM) was prolonged only by 1.4% in modern cultivars, showing an absence of positive relationship with year of cultivar release.

Table 2. Analysis of variance: A. Gain for final leaf number (FLN), phyllochron (PHY), emergence-tillering (EM-TL), tillering-stem elongation (TL-SE), stem elongation-anthesis (SE-AN), emergence-anthesis (EM-AN), anthesis-physiological maturity (AN-PM) and emergence-physiological maturity (EM-PM) of six-rowed barley with the year of cultivar release (YCR); B. Mean values for final leaf number (FLN), phyllochron (PHY), emergence-tillering (EM-TL), tillering-stem elongation (TL-SE), stem elongation anthesis (SE-AN), emergence-anthesis (EM-AN), anthesis-physiological maturity (AN-PM) and emergence-physiological maturity (EM-PM) of six-rowed barley cultivars

A

<table>
<thead>
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<th>Source of variation</th>
<th>FLN</th>
<th>PHY</th>
<th>EM-TL</th>
<th>TL-SE</th>
<th>SE-AN</th>
<th>AN-PM</th>
<th>EM-PM</th>
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<tr>
<td>Year (Y)</td>
<td>24.86</td>
<td>432.59</td>
<td>7132</td>
<td>55793</td>
<td>15324</td>
<td>197534</td>
<td>24384</td>
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<tr>
<td>Period (P)</td>
<td>4.20</td>
<td>120.86</td>
<td>160</td>
<td>60047</td>
<td>8604</td>
<td>26741</td>
<td>1131</td>
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<td>Cultivar (C)</td>
<td>1.62</td>
<td>75.21</td>
<td>1822</td>
<td>8333</td>
<td>3053</td>
<td>4049</td>
<td>1609</td>
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<tr>
<td>Y × P</td>
<td>1.14</td>
<td>6.23</td>
<td>624</td>
<td>425</td>
<td>769</td>
<td>746</td>
<td>4564</td>
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<tr>
<td>Y × C</td>
<td>0.91</td>
<td>17.48</td>
<td>1914</td>
<td>3063</td>
<td>915</td>
<td>1322</td>
<td>3332</td>
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<tr>
<td>Error</td>
<td>0.41</td>
<td>8.05</td>
<td>300</td>
<td>797</td>
<td>242</td>
<td>434</td>
<td>216</td>
</tr>
</tbody>
</table>

Correlation with YCR
Gain                             -0.02 -0.11 -0.01 -3.82 1.48 -2.35 0.19 -2.16
Gain (%)                        -0.11 -0.15 -0.00 -0.78 0.29 -0.19 0.03 -0.12
Significance                      ** ** ** ** ** **

B

<table>
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<tr>
<th>Breeding period</th>
<th>FLN</th>
<th>PHY</th>
<th>EM-TL</th>
<th>TL-SE</th>
<th>SE-AN</th>
<th>AN-PM</th>
<th>EM-PM</th>
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<tr>
<td>I</td>
<td>13.8</td>
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<td>211</td>
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<td>506</td>
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<td>560</td>
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<td>Year (Y)</td>
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<tr>
<td>2015/16</td>
<td>14.6</td>
<td>74.5</td>
<td>217</td>
<td>516</td>
<td>532</td>
<td>1265</td>
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<tr>
<td>2016/17</td>
<td>13.5</td>
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<td>199</td>
<td>467</td>
<td>506</td>
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<td>542</td>
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<tr>
<td>Mean</td>
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<td>72.3</td>
<td>208</td>
<td>491</td>
<td>519</td>
<td>1218</td>
<td>558</td>
</tr>
</tbody>
</table>

Means followed by the same letter were not significantly different at P = 0.05 by the Tukey test. ** Significant at 0.01 probability; * Significant at 0.05 probability.
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The variation in period duration from emergence to anthesis (EM-AN) was significantly influenced by Y, P, C and Y × C. Across years (Y), the duration of EM-AN varied from 1176 (IV period) to 1257 GDD (I period), with an overall average of 1218 GDD. The EM-AN period lasted longer in the first than in the second growing season. It was negatively related with year of cultivar release, with a decrease of 2.35 year⁻¹. The total crop cycle duration (EM-PM) was significantly affected by all sources of variation (Y, P, C, Y × P and Y × C). The longest duration of total crop cycle was reported in cultivars of breeding period I, while the shortest duration was observed in breeding period IV. Similar to duration period of EM-AN, the 2015/16 growing season was characterized by a longer duration of EM-PM. The total crop cycle decreased at a rate of 2.16 GDD year⁻¹.

Trait association

The PCA biplot illustrates the relationships between the means of the developmental phases, leaf appearance traits, GN, GW and GY (Figure 1B). Together, both axes accounted for 61.17% of the total variation. According to the biplot, PCA1 was mainly related to duration of the EM-PM, TL-SE and SE-AN phases, while PCA2 was mostly related to duration of AN-PM and EM-TL and GW.

Grain yield was closely related to GW, GN and duration of the SE-AN phase, while duration of PHY, EM-PM, TL-SE, EM-TL and EM-AN were negatively associated with GY. In addition, GN and duration of SE-AN were positively associated, as well as duration of grain filling period (AN-PM) and GW. The EM-AN period duration was positively related to PHY duration, while the association of EM-AN with FLN was close to zero. Moreover, EM-AN was positively associated with TL-SE, while the association with SE-AN was negative. Duration of EM-PM had a positive association with duration of EM-AN and a weak negative relationship with AN-PM. Final leaf number was negatively related to PHY.

DISCUSSION

Comparisons of phenotypic changes in representative historical sets of cultivars were widely used to study the physiological basis of yield increase (Isidro et al. 2011, Kitonyo et al. 2017). These studies were mainly focused on analyses of changes in grain yield components (Cargnin et al., 2008, Sanchez-Garcia et al. 2013) and physiological traits (Zhou et al. 2014). Genetic improvement in grain yield potential has also been associated with changes in the developmental pattern (Motzo and Giunta 2007). Since information about changes in leaf appearance traits and duration of phenological phases in the Pannonian Plain are scarce, this study examined a set of 15 six-rowed winter barley cultivars widely grown in the southern Pannonian Plain and neighboring countries over the past 50 years.

Leaf number and area are important factors influencing light interception and crop biomass at anthesis (Repkova et al. 2009, Miroslavljević et al. 2018b). Moreover, anthesis date could be represented as a function of PHY, FLN and period between flag leaf appearance and anthesis (He et al. 2012). According to the biplot presentation, duration of EM-AN was more related to PHY than FLN, while the association between PHY and FLN was negative. Although there was significant genotypic variation in FLN and PHY in our study, these traits were not significantly modified by breeding activities in the past 50 years in the southern Pannonian Plain. Similarly, no effects of oat plant breeding on leaf appearance traits were observed in oats (Peltonen-Sainio and Rajala 2007). On the other hand, Abeledo et al (2004) reported that variation in PHY was associated with YCR, while changes in FLN were not related to YCR in malting barley. Nevertheless, shorter PHY and increased FLN could be related to early vigour, leading to accelerated canopy closure, reduced evapotranspiration and improved radiation interception (Foulkes et al. 2009). Therefore, a further PHY decrease, followed by increase in FLN, could be relevant during early growth stages in environments characterized by adverse weather conditions, especially for spring small-grain barley.

The duration of the phenological phases is important for crop adaptation and yield determination under specific environmental conditions. In temperate environments, such as the Pannonian Plain, anthesis should be projected in the narrow time window between late spring frosts and early drought and temperature stress during grain filling. The results of this study showed that the pre-anthesis duration and total crop cycle are shorter in the modern than the older cultivars. Similarly, plant breeders have shortened the duration of EM-PM of modern barley compared to the oldest cultivars in Nordic environments (Rajala et al. 2017). The duration of the total plant cycle was more related to duration of the period before than after anthesis, indicating that the reduction in pre-anthesis led to a decrease in total
plant cycle. Several studies on the effect of breeding on pre-anthesis duration reported contrasting results in barley. Although the modern barley varieties had a shorter pre-anthesis period in Italy (Martintello et al. 1987), there was no clear breeding effect on the heading date of two-rowed barley cultivars in the Pannonian Plain (Grausgruber et al. 2002, Mirosavljević et al. 2016), while modern Argentinian barley cultivars tend to have a longer pre-heading period (Abeledo et al. 2003). According to previous studies, a significant decrease in pre-anthesis duration has been recorded in bread wheat (Beche et al. 2018) and durum wheat (Isidro et al. 2011). On the contrary, Mladenov et al. (2011) reported absence of significant changes in heading date in bread wheat grown in the Pannonian plain in the past century. As a result of climate change, the incidence of temperature and drought extremes during grain filling has increased during the last decades. Generally, shortening of pre-anthesis duration and physiological maturity could represent an efficient strategy to avoid unfavorable weather conditions, such as the late heat and drought stress during grain filling. Therefore, climate changes probably had a significant impact on the selection of earlier genotypes in early generations, causing a modification of the anthesis date. However, a shortening of the crop growing season decreased the level of intercepted global radiation and probably led to biomass and grain yield reduction (Trnka et al. 2015). Although our results showed a significant decrease in duration of the period from emergence to anthesis and maturity, a further shortening of these phases could be a risky strategy in breeding programs due to possible biomass and grain yield penalties. Therefore, breeders and producers should choose medium-early cultivars. Accordingly, due to the fine balance between the pre- and post-anthesis periods, medium-early cultivars are suggested for large-scale production of different cereal crops in the Pannonian Plain (Mirosavljević et al. 2018c).

Aside from the changes in duration of time to anthesis, different durations of the pre-anthesis phases, such as TL-SE and SE-AN, were also observed in our study caused by breeding activities. Generally, variations in the phenological development are the result of changes in photoperiod sensitivity, vernalization requirement and earliness per se. According to Motzo and Giunta (2007), older durum wheat cultivars had a higher photoperiod sensitivity and cold requirement than the modern ones. In our study, modern six-rowed barley cultivars tend to have shorter TL-SE and longer SE-AN periods. A prolongation of the stem elongation phase could be important to improve floret survival, leading to a higher spike dry weight at anthesis and increase in grain number per spike (Slafer 2003, González et al. 2011). Furthermore, the results of our study indicated that the SE-AN duration was positively related with GN and GY. Accordingly, efforts to further increase SE-AN duration, without modifying the anthesis date should be included as an important strategy in cereal breeding programs, as previously noted by Miralles and Slafer (2007).

No systematic modification of the length of the grain filling period as a result of barley breeding in the past 50 years was observed in the six-rowed cultivars grown in the Pannonian Plain. The present study indicated that GW was closely related with duration of AN-PM. An earlier anthesis date shifted the grain filling period towards better environmental conditions, resulting in a GW increase in the modern barley cultivars (data not shown). These findings contradict those previously reported (Serrago et al. 2013, Mirosavljević et al. 2018a), which suggested that the photosynthesis assimilation rate is more important than grain filling duration.

CONCLUSIONS

As a result of breeding activities in the Pannonian Plain over the past 50 years, the anthesis date and total plant cycle in six-rowed barley cultivars were reduced. The shortening of the EM-AN period was the result of the shorter TL-SE phase, while SE-AN has been positively associated with YCR. The duration of SE-AN was positively related with GN and that of AN-PM with GW. There was no association between leaf appearance traits and YCR. Therefore, breeding efforts with six-rowed barley in the Pannonian Plain led to the development of modern high-yielding cultivars, characterized by prolonged SE-AN and shortened TL-SE, EM-AN and EM-PM periods, but did not affect FLN and PHY. Further fine adjustments in the duration of pre- and post-anthesis phases, which are related to GN and GW determination, should be included in breeding programs as an important strategy for grain yield improvement in six-rowed winter barley.

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