

Effects of the *Blp1* locus, which controls melanin accumulation in the barley ear, on the size and weight of seeds

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Влияние локуса *Blp1*, контролирующего синтез меланина в колосе ячменя, на размер и вес зерна

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Background. In cereals, photosynthetically active parts of the ear significantly contribute to seed size and weight at the grain-filling stage. In barley, ear tissues may accumulate melanin pigments synthesized in chloroplast-derived melanoplasts. Effects of such pigments on yield parameters of seeds have not been evaluated to date.

Materials and methods. Seed weight and size assessed by image analysis were compared between two near-isogenic barley lines differing in alleles of the *Blp1* gene, which determines melanin accumulation in ear tissues. Data on grain-related parameters were collected during 6 years and include data on seeds grown either in the field or under greenhouse conditions.

Results and discussion. A negative effect of the *Blp1* locus on the weight of 1000 seeds harvested in the field but not in the greenhouse was revealed. To determine whether this effect is related to grain size, a comparison of two-dimensional linear parameters of seeds between the lines was performed. It was shown that unlike the length and the area of seeds, the width of seeds was also negatively affected by the *Blp1* locus. Although the same factors affected the weight of 1000 seeds and the width of seeds, a correlation between them was not found, implying a dependence of seed weight on other factors such as thickness and its related parameter, seed volume.

Conclusion. Effects of barely ear pigmentation and of the gene controlling it on yield-related parameters of seeds were studied here for the first time. The observed negative impact of the *Blp1* locus on seed weight and size may be mediated by an interfering chloroplast activity and/or accumulation of assimilates via melanogenesis. Additional studies are necessary to test this supposition and to investigate the interaction of melanin synthesis and photosynthetic activity of the tissues accumulating this pigment.

Key words: *Hordeum vulgare* L., melanoplast, photosynthesis, assimilate, grain filling.

Актуальность. Фотосинтезирующие ткани колоса ячменя являются источниками ассимилирующих веществ, которые вносят большой вклад в формирование размера и массы зерна. У ячменя в тканях колоса могут накапливаться меланиновые пигменты, влияние которых на показатели урожайности зерна исследованы не были.

Материалы и методы. В работе было проведено сравнительное исследование массы и линейных размеров зерна, полученных с помощью анализа изображений, почти изогенных линий ячменя, отличающихся по локусу *Blp1*, контролирующему синтез меланиновых пигментов в тканях колоса ячменя – в цветковой чешуе, перикарпии зерна и осях.

Результаты и обсуждение. Было показано, что в полевых условиях локус *Blp1* отрицательно влияет на массу 1000 зерен. Чтобы определить обусловлен ли наблюдаемый эффект уменьшением размеров зерна, было проведено сравнение линейных параметров зерна между линиями. Было показано, что, в отличие от длины и площади семян, локус *Blp1* отрицательно влияет на ширину семян. Несмотря на то что на массу и ширину зерна влияли одни и те же факторы, корреляции между этими параметрами выявлено не было, что, возможно, связано с зависимостью массы зерна от других параметров, например толщины и связанного с ней объема зерна.

Заключение. Впервые было проведено исследование роли пигментов и контролирующих их формирование генов на показатели продуктивности зерна. Обнаруженный отрицательный эффект локуса *Blp1* на массу и ширину зерна может быть связан с нарушениями фотосинтетической активности и накоплением ассимилятов в зерне, вызванных синтезом меланинов в тканях колоса. Для проверки выдвинутого предположения и установления характера связи между меланогенезом и фотосинтезом необходимы дополнительные исследования.

Ключевые слова: *Hordeum vulgare* L., меланопласты, фотосинтез, ассимиляты, налив зерна.

Introduction

The size and weight of barley grains are agronomically important traits that significantly contribute to the final yield. These traits are determined by linear parameters of seeds: length, width, and thickness as well as the degree of grain filling (Zhang et al., 2012). They are considered the primary traits for crop domestication and artificial breeding. Modern barley varieties have larger grains as compared to their ancestors (Hughes et al., 2019). Although grain size and weight have a favorable influence on seedling vigor and yield, including malt yields and feed production capacity, little is known about the genes that determine these parameters in barley. Some attempts have been made to clarify the genetic control of these traits. Quantitative trait loci and hotspots for grain size and weight are reported to be distributed on all seven chromosomes (Ayoub et al., 2002; Walker et al., 2013; Wang et al., 2019). Additionally, several genes have been identified that affect barley grain size or weight, e.g., *Nud* (Taketa et al., 2008), *Int-c* (Ramsay et al., 2011), *Vrs1* (Sakuma et al., 2017), *Vrs2* (Youssef et al., 2017), *Vrs3* (Bull et al., 2017), and *Vrs4* (Koppolu et al., 2013).

Besides the aforementioned genetic factors, grain yield parameters in cereals are influenced by plant nutrition during the grain-filling stage. Photosynthetically active organs of the ear such as lemmas, paleas, glumes, and awns may significantly contribute to grain size and weight (Brazel, Ó'Maoiléidigh, 2019). For example, in wheat, the ear contributes 45–65% of grain filling whereas the rest of the above-ground parts of the plant contribute 35–69% (Sanchez-Bragado et al., 2016). Although awn length not always contributes to an enhanced grain yield, reduced awn size correlates with a reduction in individual grain size and an increased number of shriveled grains (Rebetzke et al., 2016).

Some specimens of barley can accumulate melanin pigments in such ear tissues as the grain pericarp, husks, and awns (Shoeva et al., 2020). This trait is under the control of the *Blp1* (*Black lemma and pericarp 1*) locus mapped to chromosome 1HL (Long et al., 2019). Because melanin accumulates at the grain-filling stage, which is crucial for grain size development, this pigment is assumed to affect grain yield-associated traits. To test the impact of the *Blp1* locus on the size and weight of barley (*Hordeum vulgare* L.) seeds, a comparative analysis of these traits between two near-isogenic lines (NILs) having different alleles of the *Blp1* locus was carried out in this study.

Material and methods

Plant material and growth conditions

Spring barley cv. 'Bowman' ('Bowman From Fargo', NGB22812, www.nordgen.org) and its NIL i:Bw*Blp1* (NGB20470) developed for the *Blp1* locus controlling melanin accumulation in spike tissues – the grain pericarp, husks, and awns (Figure) (Druka et al., 2011) – were grown either in a greenhouse of the ICG SB RAS (Novosibirsk, Russia) under a 12 h photoperiod in a temperature range of 20–25°C in autumn (from October to January) or spring (from February to May) vegetation seasons or in the experimental field of the ICG Breeding Genetic Complex (Novosibirsk, N54.847102, E83.127422). During 2015–2020, seeds of both lines were collected and subjected to the comparative analysis. In total, data on 14 trials (seed weight measurement; each trial corresponds to a distinct vegetation season and study site; Table 1) and 12 trials (measurement of all other parameters; Table 2) were collected including data on 6-year observations of the plants under field conditions and 4- or 3-year observations in autumn and spring vegetation seasons in the greenhouse.



Figure. Ears of 'Bowman' (top) and its NIL i:Bw*Blp1* (bottom) at the full-maturity stage
Рисунок. Колос ячменя сорта 'Bowman' (сверху) и его почти изогенной линии i:Bw*Blp1* (снизу) на стадии полной спелости зерна

Table 1. The weight of 1000 seeds in 'Bowman' and i:BwBlp1**Таблица 1. Показатели массы 1000 зерен сорта 'Bowman' и линии i:BwBlp1**

Year	Growth conditions	Bowman	i:BwBlp1	p-value	Significant difference
2015	field	58.7 ± 0.8	53.0 ± 0.3	0.0495	yes
	greenhouse, S	47.1 ± 0.1	45.7 ± 2.2	0.5127	no
2016	field	58.6 ± 0.7	50.6 ± 2.7	0.0495	yes
	greenhouse, A	54.4 ± 0.8	41.4 ± 0.8	0.0495	yes
2017	field	57.1 ± 1.0	57.0 ± 0.3	0.8273	no
2018	field	49.6 ± 0.6	47.4 ± 0.3	0.0495	yes
	greenhouse, S	44.7 ± 0.7	44.0 ± 0.2	0.1840	no
	greenhouse, A	46.6 ± 1.2	47.2 ± 0.3	0.8222	no
2019	field	47.3 ± 0.1	45.4 ± 0.4	0.0431	yes
	greenhouse, S	46.0 ± 0.7	53.4 ± 0.7	0.0495	yes
	greenhouse, A	36.2 ± 1.3	39.4 ± 1.5	0.0495	yes
2020	field	48.1 ± 0.7	47.5 ± 0.5	0.5127	no
	greenhouse, S	49.4 ± 1.7	45.0 ± 1.1	0.0495	yes
	greenhouse, A	37.9 ± 0.1	44.6 ± 0.0	0.0339	yes

Note: mean of three biological replicates ± standard deviation, in grams; differences are significant at $p \leq 0.05$, U test; A: autumn; S: spring
 Примечание: среднее значение трех биологических повторений ± стандартное отклонение, в граммах; различия значимы при $p \leq 0.05$, U -критерий Манна-Уитни; A: осенняя вегетация; S: весенняя вегетация

Table 2. The linear parameters and weight of 30 seeds in 'Bowman' and i:BwBlp1**Таблица 2. Линейные размеры и масса 30 зерен сорта 'Bowman' и линии i:BwBlp1**

Year	Growth conditions	Parameter	Bowman	i:BwBlp1	p-value	Significant difference
2015	field	length, mm	7.50 ± 0.25	7.59 ± 0.16	0.6015	no
		width, mm	3.43 ± 0.11	3.07 ± 0.05	0.0086	yes
		area, mm ²	19.48 ± 1.20	17.97 ± 0.62	0.0163	yes
		mass, g	1.76 ± 0.04	1.56 ± 0.05	0.0082	yes
2016	field	length, mm	7.79 ± 0.25	7.67 ± 0.11	0.2506	no
		width, mm	3.28 ± 0.09	3.12 ± 0.06	0.0163	yes
		area, mm ²	19.70 ± 1.00	18.41 ± 0.10	0.1172	no
		mass, g	1.59 ± 0.07	1.53 ± 0.05	0.1161	no
2017	field	length, mm	7.51 ± 0.19	8.06 ± 0.23	0.0163	yes
		width, mm	3.15 ± 0.03	3.20 ± 0.07	0.1172	no
		area, mm ²	18.31 ± 0.55	19.85 ± 0.90	0.0163	yes
		mass, g	1.47 ± 0.03	1.65 ± 0.07	0.0088	yes
2018	field	length, mm	9.89 ± 0.28	9.27 ± 0.35	0.0283	yes
		width, mm	3.67 ± 0.10	3.38 ± 0.10	0.0090	yes
		area, mm ²	27.49 ± 1.57	23.89 ± 1.41	0.0283	yes
		mass, g	1.51 ± 0.05	1.44 ± 0.02	0.0350	yes

Table 2. The end
Таблица 2. Окончание

Year	Growth conditions	Parameter	Bowman	i:BwBlp1	p-value	Significant difference
2018	greenhouse, S	length, mm	7.44 ± 0.82	8.00 ± 0.51	0.1172	no
		width, mm	3.33 ± 0.39	3.36 ± 0.13	0.9168	no
		area, mm ²	18.86 ± 4.45	20.46 ± 1.98	0.7540	no
		mass, g	1.39 ± 0.04	1.33 ± 0.04	0.0356	yes
	greenhouse, A	length, mm	7.87 ± 0.76	8.56 ± 0.19	0.0283	yes
		width, mm	3.50 ± 0.36	3.66 ± 0.12	0.2506	no
		area, mm ²	21.00 ± 3.83	24.07 ± 1.21	0.0472	yes
		mass, g	1.42 ± 0.02	1.46 ± 0.03	0.0555	no
2019	field	length, mm	9.18 ± 0.10	8.67 ± 0.34	0.0163	yes
		width, mm	3.40 ± 0.12	3.30 ± 0.13	0.1172	no
		area, mm ²	23.66 ± 1.08	21.91 ± 1.78	0.1172	no
		mass, g	1.44 ± 0.03	1.35 ± 0.04	0.0278	yes
	greenhouse, S	length, mm	7.97 ± 0.13	9.45 ± 0.20	0.0090	yes
		width, mm	3.63 ± 0.11	3.53 ± 0.04	0.1172	no
		area, mm ²	21.46 ± 0.82	25.48 ± 0.65	0.0090	yes
		mass, g	1.39 ± 0.09	1.60 ± 0.04	0.0090	yes
	greenhouse, A	length, mm	7.71 ± 0.34	7.72 ± 0.47	0.7540	no
		width, mm	3.37 ± 0.21	3.09 ± 0.20	0.0758	no
		area, mm ²	19.49 ± 2.00	18.35 ± 2.27	0.6015	no
		mass, g	1.12 ± 0.05	1.19 ± 0.03	0.0723	no
2020	field	length, mm	8.44 ± 0.35	8.03 ± 0.47	0.0758	no
		width, mm	3.58 ± 0.13	3.39 ± 0.17	0.0758	no
		area, mm ²	23.05 ± 1.77	20.86 ± 2.38	0.1172	no
		mass, g	1.44 ± 0.04	1.42 ± 0.06	0.2933	no
	greenhouse, S	length, mm	8.34 ± 0.95	8.03 ± 0.35	0.1172	no
		width, mm	3.60 ± 0.26	3.39 ± 0.20	0.1172	no
		area, mm ²	22.32 ± 3.87	20.85 ± 2.02	0.1745	no
		mass, g	1.50 ± 0.06	1.38 ± 0.03	0.0090	yes
	greenhouse, A	length, mm	7.78 ± 0.47	7.73 ± 0.45	0.7540	no
		width, mm	3.36 ± 0.13	3.22 ± 0.12	0.0758	no
		area, mm ²	19.40 ± 1.80	19.21 ± 1.91	0.9168	no
		mass, g	1.15 ± 0.05	1.31 ± 0.04	0.0090	yes

Note: mean of five biological replicates ± standard deviation; differences are significant at $p \leq 0.05$, U test; A: autumn; S: spring

Приложение: среднее значение пяти биологических повторений ± стандартное отклонение; различия значимы при $p \leq 0.05$, U -критерий Манна-Уитни; A: осенняя вегетация; S: весенняя вегетация

Evaluation of grain yield-associated traits

The weight of 1000 seeds was measured in triplicate (three biological replicates) in each trial. Linear parameters of seeds (length, width, and area) were measured in five biological replicates with 30 seeds per replicate using the Seed-Counter application for Android (Komyshev et al., 2017). The same 30 seeds were weighed in each replicate. The significance ($p \leq 0.05$) of differences in parameters between the two lines was assessed by the nonparametric Mann-Whitney U test. The nonparametric Kruskal-Wallis H test was performed for determining the influence of factors “growth conditions” (greenhouse or field), “year” (field conditions in a distinct year) and “genotype” (‘Bowman’ or i:BwBlp1) on seed size parameters. Correlations between the linear parameters of seeds and their weight were evaluated by means of Spearman’s rank correlation coefficient. Statistical analysis of the resulting data was carried out in Statistica v.6.1 (StatSoft Inc., 2004).

Results

The weight of 1000 seeds

These data were collected for the NILs grown under the field or greenhouse conditions during 6 consecutive years (Table 1). The parameter was within ranges 36.2–58.7 and 39.4–57.0 g in ‘Bowman’ and i:BwBlp1, respectively. ‘Bowman’ was found to have heavier seeds in comparison with the i:BwBlp1 line in 10 of 14 trials, and in six of them, the differences in the weight of 1000 seeds were statistically significant including four field trials (Table 1).

One-way ANOVA on ranks was conducted to evaluate the effects of different factors on the weight of 1000 seeds (Supplementary Materials, Table S1)¹. It was revealed that growth conditions and the year significantly affected this parameter when assessed either in the whole dataset of 14 trials or in the datasets of ‘Bowman’ and i:BwBlp1 separately. We showed that on average, both genotypes have heavier seeds when harvested from the plants grown under field conditions in comparison with those grown in the greenhouse (Supplementary Materials, Table S2). Under the field conditions, the genotype affected the weight of 1000 seeds significantly. Independent on years being compared ‘Bowman’ seeds harvested from the plants grown under the field conditions were on average heavier than i:BwBlp1 seeds (Supplementary Materials, Table S3). Under the greenhouse conditions, the influence of factor genotype on this parameter was not detectable in the combined dataset of spring and autumn vegetation seasons and in the separate datasets of these vegetation seasons. In dependence on years being compared, greenhouse-harvested ‘Bowman’ seeds were more often either heavier or lighter in weight and in some cases did not differ from greenhouse-harvested i:BwBlp1 seeds (Supplementary Materials, Table S3).

Linear parameters of seeds

The length of seeds varied within ranges 7.44–9.89 and 7.59–9.45 mm in ‘Bowman’ and i:BwBlp1, respectively (Table 2). ‘Bowman’ was found to have longer seeds in comparison with the i:BwBlp1 line in 6 of 12 trials, and in two of them, the differences in the length of seeds were statistically significant, while in three trials, i:BwBlp1 had significantly longer seeds in comparison with ‘Bowman’. In 7 of 12 trials, the differences in the seed length between the lines were not significant. Factors growth conditions and genotype did not affect this parameter, while the year did, both in ‘Bowman’ and in

i:BwBlp1 (Supplementary Materials, Table S1). In dependence on years being compared, the seeds of both genotypes either were longer or did not differ when harvested from the plants grown under the field conditions in comparison with those grown in the greenhouse. In a few cases only, the seeds harvested in the greenhouse were longer in comparison with those harvested under the field conditions (Supplementary Materials, Table S4). In dependence on years being compared, ‘Bowman’ seeds were either longer or shorter or did not differ from i:BwBlp1 seeds when harvested in the field, but there were no ‘Bowman’ seeds longer than BwBlp1 seeds when harvested in the greenhouse (Supplementary Materials, Table S5).

The width of seeds was within ranges 3.15–3.67 and 3.07–3.66 mm in ‘Bowman’ and i:BwBlp1, respectively (Table 2). The seeds harvested from ‘Bowman’ plants were wider than i:BwBlp1 seeds in 9 of 12 trials, and in three of them, the differences were statistically significant (Table 2). Unlike the length of seeds, the width was influenced by the growth conditions, year, and genotype (Supplementary Materials, Table S1). For both lines, grains were either wider or did not differ when harvested from plants grown in the greenhouse than in the field; only in a few cases, the seeds harvested in the field were wider than those harvested in the greenhouse (Supplementary Materials, Table S6). In dependence on years being compared, Bowman seeds were more often wider than i:BwBlp1 seeds when harvested in the field, while they did not differ more often from i:BwBlp1 seeds harvested in the greenhouse; in a few cases only, i:BwBlp1 seeds were wider than ‘Bowman’ seeds (Supplementary Materials, Table S7).

The area of seeds varied within ranges 18.31–27.49 and 17.97–25.48 in ‘Bowman’ and in the i:BwBlp1 line, respectively. ‘Bowman’ was found to have larger seeds in comparison with the i:BwBlp1 line in eight of 12 trials, and in two of them, the differences were statistically significant (Table 2). As in the case of seed length, the growth conditions and genotype did not affect seed area, while the year did, both in ‘Bowman’ and in i:BwBlp1 (Supplementary Materials, Table S1). In dependence on years being compared, field-harvested ‘Bowman’ seeds were more often either larger or did not differ from greenhouse-harvested seeds, while BwBlp1 seeds harvested in the greenhouse were more often either larger or did not differ from seeds harvested in the field (Supplementary Materials, Table S8). ‘Bowman’ seeds were more often larger or did not differ from i:BwBlp1 seeds when harvested in the field, while ‘Bowman’ seeds were more often smaller or did not differ from i:BwBlp1 seeds when harvested in the greenhouse (Supplementary Materials, Table S9).

A moderate positive correlation between the length and width of seeds ($r_s = 0.70$, $p < 0.0001$) was revealed. Seed area positively correlated with seed length ($r_s = 0.95$, $p < 0.0001$) and width ($r_s = 0.86$, $p < 0.0001$), while seed weight did not show associations with any linear parameters of the seeds.

Discussion

Plant melanin can accumulate in covering tissues of seeds, where its role is not obvious at present. According to summarized data reviewed by Glagoleva et al. (2020), melanin is not essential for plants, but under some conditions, it can give them some advantages. For instance, melanin can confer more vigor on seeds, as in brown seeds of watermelon, which are heavier and have higher germination and emergence percentages and seedling fresh and dry weights relative to light-colored seeds (Mavi, 2010). There have been attempts to determine the function of melanin pigments accumulating in

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barley seeds during exposure to salinity, drought, or cadmium toxicity in the same genetic model of NILs differing in grain color that are used in the current study. The results indicated that melanin does not give any advantages to barley seedlings under the stressful conditions tested (Glagoleva et al., 2020).

Recently, the localization of the melanin pigment in aging chloroplasts of pericarp and husk tissues was identified in barley seeds (Shoeva et al., 2020). Dynamics of melanin pigmentation development coincided with the grain-filling stage. This finding suggests that melanin can affect photosynthetic activity of ear tissues accumulating it and, as a consequence, some parameters of seeds.

To identify a possible effect of melanin itself or the gene that determines its accumulation on grain weight and size, a comparative study of two NILs differing in grain color was carried out here. Datasets on the weight of 1000 seeds and linear parameters of seeds were collected, respectively, for 14 and 12 trials (Tables 1, 2, respectively) including 6-year observations on the seeds harvested in the field.

The observed impact of growth conditions in a given year on seed weight and size in both studied genotypes ('Bowman' and its NIL i:BwBlp1) was an expected one, while the effect of the *Blp1* locus (controlling melanin accumulation in the barley ear) on these parameters was shown for the first time. The negative effect of melanin accumulation in ear tissues on the weight of 1000 seeds was small and depended on growth conditions: it was more pronounced in seeds harvested in the field but not in the greenhouse. To determine whether this effect on weight is related to grain size, a comparison of two-dimensional linear parameters of seeds between the NILs was performed here by image analysis.

Unlike seed length and area, the width of seeds was affected by factor genotype. The parameter was on average greater in noncolored 'Bowman' than in the black-grained line. Even though the same factors affected the weight of 1000 seeds and seed width, there was no correlation between these parameters. Moreover, seed weight did not correlate with any two-dimensional linear parameters of seeds (length, width, and area of seeds), while there were strong positive correlations among the linear parameters. Earlier, a dependence of the volume of wheat and barley seeds on the death and to a lesser extent on the width of seeds was demonstrated (Hughes et al., 2019). Different contributions of morphometric parameters to seed shape and as a consequence to seed volume and weight may explain the absence of associations between seed weight and the two-dimensional parameters of seeds assessed here.

The size of seeds is determined by the accumulation of assimilates originating from photosynthetically active tissues of plants, including ear tissues such as the husk and awns (Brazel, Ó'Maoiléidigh, 2019). As a possible explanation of the negative impact of melanogenesis on the weight and size of barley seeds, one can consider declining photosynthetic processes in chloroplasts of the ear tissues accumulating melanins. This notion is supported by downregulation of photosynthesis-related genes in the melanin-accumulating i:BwBlp1 line in comparison to control 'Bowman' plants as evidenced by high-throughput RNA sequencing (Glagoleva et al., 2017).

Conclusion

For the first time, attempts were undertaken to evaluate the influence of pigmentation and of the gene controlling it on yield-related parameters of seeds such as weight and size. Some negative effects of melanin presence in ear tis-

sues on the weight of 1000 seeds and their width were identified. The negative effect of the *Blp1* locus on seed weight and size did not manifest itself in all trials (i.e., vegetation seasons and study sites) and was dependent on growth conditions in different years. The observed negative impact of the *Blp1* locus on seed weight and size may be mediated by an interfering chloroplast activity and/or by the accumulation of assimilates via melanogenesis. Additional research is necessary to investigate the interaction of melanin synthesis and photosynthetic activity of the tissues accumulating this pigment.

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