

1 **Prospective use of barley spike/flower homeotic single and double mutants for ornamental**
2 **purposes**

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16

17 **Abstract**

18 Barley and other cereals only in small portions are used as ornamentals for their attractive spike
19 structure and other features, and thus, there is a need to enhance approaches for the enrichment of their
20 ornamental genetic resources. Homeotic *Hooded* (*Hd*) mutants, caused by a 305-bp duplication in intron
21 IV of the *BKn3* gene and characterized by ectopic flowers instead of awns, are attractive ornamental
22 plants. However, it has been proposed that the hybridization of *Hd* mutants with other homeotic mutants
23 may widen resources of attractive forms. Two groups of lines were studied: those resulting from the
24 hybridization of *Hooded* in cv. Colsess II with other *Hd* mutants and those resulting from the
25 hybridization of *Hd* with other homeotic mutant *tweaky spike₂* (*tw₂*). *tw₂* has lodicules that are irregularly
26 transformed to reproductive organs and specific spike shapes. Attractive lines were selected, propagated
27 and studied in distinct generations (corresponding to F₉₋₁₅) for decorative quality and quantitative
28 characters. Variations of vegetation time, weight of grains, resistance to lodging and diseases, protein
29 and starch contents are presented. In total, 231 lines were analysed. Not all selected attractive lines
30 possess the mutant *BKn3* allele. Wider and more attractive spectra were revealed when different types of
31 mutants, *Hd* and *tw₂*, were hybridized. More attractive lines were selected among the offspring of *tw₂* ×
32 *Lh* and *tw₂* × *Br*. Several lines are also valuable for high protein content.

33 **Keywords** ornamental barley, homeotic mutants, *BKn3* gene, hybridization, line variations, quantitative
34 characters

35

36 Introduction

37 Attractive panicle/spike structure and other features, such as stem and grain form and colour,
38 variegated leaves, and the possibility for making dry bouquets, allow many grasses (*Poaceae*), including
39 agricultural plants such as wheat, barley, rye, and oats, to be used for ornamental purposes. In several
40 countries (Germany, Finland, Lithuania, Latvia, Slavian and other countries), folk art formed with a
41 treated tied cereal straw, named Gardens, Puzuri, Saulites, Himmeli, Solomennyj pauk (Straw Spider),
42 and Pajaki, are common (<https://www.pinterest.com/balticsmith/puzuri-un-saul%C4%ABtes-himmeli-%C5%A1iaud%C5%B3-sodai-%D1%81%D0%BE%D0%BB%D0%BE%D0%BC%D0%B5%D0%BD%D0%BD%D1%8B%D0%B9/>). In the Vilnius region of Lithuania, ‘palm’ bouquets (called ‘verbos’) are very popular during the
44 Kaziukas Fair (comparable to St. Casimir name day) and on Easter (Supplementary Fig. S1). Even though
45 ornamental barley occupies a small economic niche, employment in the preparation and realization of
46 ornamental plants utilizes significant human resources, and improvements of ornamental barley genetic
47 resources are necessary.

48 Several barley (*Hordeum vulgare* L.) varieties and forms are on the market as ornamentals due to
49 their exclusive spike or grain features: Tibetan Purple Hulless, Arabian Blue, and Blackheart are known
50 for blue/dark spikes and grains; Mongold for unusual striped yellow spikes; Bearded for bearded awns
51 that make wonderful, dried arrangement materials; Sheba for long golden awns; and others. Blends of
52 several varieties are also offered. Variegated forms of barley are also popular, which are used not only
53 as ornamental plants but also as an attractive diet for cats (named Cat grass – several varieties are known
54 as Taby Cat, Variegated and others).

55 Years ago, attention was paid to the exotic beauty of barley mutants with an abnormal spike
56 structure (Martini and Harlan 1942), which are currently known as homeotic, in which one inflorescence
57 (spike)/flower element is converted into another (Sommer et al. 1990; Druka et al. 2011; Stanca et al.,
58 2013). However, these ideas were not realized for years (Siuksta et al. 2012). Considered especially
59 decorative are the dominant barley mutants *Hooded* (*Hd*), which are characterized by an ectopically
60 developed extra flower with inverted polarity, instead of the lemma awn or on the awns, and caused by
61 a 305-bp duplication in intron IV of the *BKn3* gene (Müller et al. 1995). The *BKn3* gene is a
62 representative of Class I of the KNOX family homeobox genes that are mainly required for meristem
63 development and differentiation, and for the initiation of inflorescence and floral meristems (Bolduc et
64 al. 2012; Richardson et al. 2016). Lack of awns and a thinner wax layer *Hd* barley make it suitable for
65 forage, and several barley cultivars (Haybet, Hays, Dillon, Lavina) were developed for forage from *Hd*
66

68 mutant types (Bowman et al. 1994; Hadjichristodoulou 1994; Jacob and Pescatore 2012). However, no
69 steps to use *Hd* barley mutants for ornamental purposes had been made until our preliminary publication
70 on the use of homeotic *Hooded* barley mutants and their segregation products, derived from the
71 hybridization of *Hd* mutants with the other recessive homeotic mutant *tweaky spike₂* (*tw₂*) (Siuksta et al.
72 2012). Only years later were *Hooded* mutants (named *Hooked*) included in a few market lists.

73 Recessive pleiotropic *tweaky*-type barley mutants have long been known (Reid and Wiebe 1968;
74 Jensen and Jørgensen 1975; Bieliūnienė et al. 2003) but have yet to be fully genetically characterized.
75 The *tweaky spike₂* (*tw₂*) mutants are not as imposing as the *Hd* mutants. They have a specific spike
76 structure and lodicules that are irregularly transformed into stamens, pistils and/or chimeric organs. Short
77 naked gaps endow the spikes with an interrupted, tweaked form that is associated with an imbalance of
78 the auxin pathway (Šiukšta et al. 2015, 2018).

79 The aim of the present study was to show that the hybridization of *Hd* mutants or with the other
80 homeotic mutant *tweaky spike* may significantly broaden the resources of attractive forms, although the
81 *Hooded*-type barley homeotic mutants, with awn transformation to flower-like structures, are very
82 attractive themselves and may be used directly for ornamental purposes. Attractive barley lines, resulting
83 from the segregation of hybrids, are characterized.

84

85 **Materials and methods**

86

87 *Plant materials*

88 The dominant *Hooded*-type barley mutants *Hooded/Kap1.a* in cv. Colless II (GSHO 67, hereafter
89 referred to as *H*), *Lemma hooded* (*Lh*, GSHO 932), which possesses a phenotype that is very unique for
90 *Hooded*-type mutants, *Multiflorous* (*Mf*, GSHO 79), *Hoods on center spikelet*, (*Hcs*, GSHO 666), *Dense*
91 *wing hood* (*Dwh*, GSHO 928), and *Brittle rachis* (*Br*, GSHO 1565) were obtained from the USDA-ARS
92 National Small Grains Collection (Aberdeen, ID, USA) (Figs. 1 and 2). The recessive allelic mutant
93 *tweaky spike₂* (*tw₂*) was induced by chemical mutagenesis using ethylene imine from barley cv. Auksiniai
94 II, which in Vilnius trials was used as the *wild type* (Supplementary Fig. S3) because it has the same
95 genetic background as the mutant *tw₂*.

96

97 *Hybridization conditions*

98 Paternal single mutants were hybridized in 2003 (Vaitkūnienė et al. 2004). In the combination of
99 *tw₂* with *Hd*, the recessive mutant *tw₂* was used as the mother plant, allowing control of the hybridization

100 process because all the *Hd* mutants used in the present study are dominant, whereas the expression of the
101 recessive *tw₂* phenotype indicates homozygosity for the *tw₂* mutation. When different *Hd* mutants were
102 hybridized with themselves, the *Hooded* in cv. Colseess II mutant was used as a mother plant. Beginning
103 in 2009 (using initial F₆ plants), individual lines were created, each initially from a separate plant, and
104 selection according to ornamental spike quality was initiated. At present, 231 ornamental lines are in the
105 collection.

106

107 *Plant growing conditions and quantitative trait analysis*

108 All lines were grown in the Botanical Garden of Vilnius University, but quantitative trait studies
109 of ornamental lines were performed in Vilnius and at the Institute of Agriculture of the Lithuanian
110 Research Centre for Agriculture and Forestry (Dotnuva, Akademija) according to the technology used
111 for breeding from 2012–2018. The soil of the experimental site was Endocalcari – Epihypogleyic
112 Cambisol (CMg-n-w-can) with close to neutral acidity, moderately supplied with available phosphorus
113 and potassium, containing 2.25 g kg⁻¹ humus. Grain legumes (pea) was used as the pre-crop. The
114 experimental plot size was 1.5 m wide and 10 m long, with a plant density of 450 grains per m². The
115 trials were arranged in a randomized complete block design with two replications. Standard agricultural
116 practices were conducted in all seasons. Fertilizer NPK rates before sowing were 90-60-60 kg ha⁻¹.
117 Herbicides and insecticides were applied as appropriate. Weather data, collected from the meteorological
118 station located at Dotnuva, are shown in Supplementary Table S1. The growing duration date (GD) was
119 calculated as the number of days from germination to the physiological maturity date (BBCH 89) and
120 were recorded for each plot when 50% of the plants reached this stage. Yield data were adjusted to 15%
121 moisture content. The thousand grain weight (TGW) (g) was determined from measuring two sets of 500
122 grains per plot and expressed as the weight of 1000 grains with the CONTADOR Seed counter. The
123 hectolitre weight (HLW) (kg hl⁻¹) was determined by measuring a known volume of the natural seed
124 sample with a PFEUFFER Hecto grain tester. A sample was taken from each plot before harvesting to
125 measure the length of individual plants and productivity components. For the study of quantitative
126 characters, 30 plants or spikes were analysed in each line. Diseases were assessed at medium milk
127 development stage (BBCH 75). The resistance to disease and lodging was measured at points using a
128 1–9 scale: for diseases, point 1 – no visible symptoms of disease, point 9 – plants heavily infected
129 (infection ≥ 80%); for lodging resistance, point 1 – plants very inclined to lodging, 9 – all plants erect.
130 Resistance to lodging was evaluated from flowering to harvest.

131

132 *Grain chemical composition analysis*

133 Grain quality characters were determined in dry material. Protein and starch content were
134 evaluated using standard methods in the Chemical Research Laboratory of Institute of Agriculture. Total
135 starch content was determined by hydrochloric acid dissolution (ICC 123/1), and protein content was
136 determined by the Kjeldahl method (LSTEN ISO 20483) from 2013–2015 and by using an Infratec™
137 1241 Grain Analyser (FOSS, Analytical A/S, Denmark) from 2016–2018.

138
139 *Molecular analysis of a 305-bp duplication in the BKn3 gene*

140 The presence of a 305-bp duplication in the *BKn3* gene in the tested lines was determined
141 electrophoretically based on the size of the amplified fragments, as described by Šiukšta et al. (2018). A
142 previous study (Šiukšta et al. 2018) has shown that cv. Auksiniai II, considered a *WT*, and *tw2* carry the
143 European *BKn3* allele I (*k*), while all *Hd* single and real double mutants carry allele IIIc (*K*), what was
144 determined according to Badr et al. (2000).

145
146 *Statistical analysis*

147 The data were statistically processed with a software package using ANOVA (Tarakanovas and
148 Raudonius 2003) and statistically analysed using Fisher's analysis of variance. The least significant
149 difference test at a 0.05 probability level was employed to compare differences among the treatment
150 means. The significance values of the mean comparisons are presented in Supplementary Tables S2–S5.

151
152 **Results**

153 *Common characteristics of ornamental barley lines*

154 The ornamental value of barley comprises not only the easily determined morphological/qualitative
155 and quantitative spike characters, such as row number, colour of immature and mature plants (including
156 straw and ripened grain colour), and awn length (especially awn length in relation to spike length) but
157 also the common spike impression (Figs. 1 and 2, Supplementary Figs. S2, S3, S5, S6).

158 As mentioned in the Introduction, barley *Hooded* mutants are considered ornamental. Especially
159 attractive and unique among *Hd* mutants is the *Lemma hooded* mutant. Its ectopic floral structures (EFS)
160 occur on long awns, whereas the other *Hd* mutants are awnless, and the contrasting *Hd* mutants, used for
161 hybridization, express EFS to various extents (Figs. 1 and 2).

162 In general, variation spectra according to ornamental value are wider among segregation products
163 in all hybrid combinations (Table 1, Supplementary Tables S2 and S3, Figs. 1 and 2, Supplementary

164 Figs. S2, S3). However, not only variations of EFS but also other spike features, caused by different
165 genetic backgrounds, form impressions of selected ornamental lines. This is especially clear for lines
166 derived from the hybridization of *H* with other *Hd* mutants. Six-rowed lines are more attractive. The
167 majority of lines selected as ornamental in this group comprise six-row barley, including lines selected
168 from the *H* × *Br* cross-combination, in which *Br* possesses a dominant character: the two-row spike.

169 When two different mutant genes, *Hd* and *tw₂*, are introduced into the hybridization, variation
170 spectra were much wider not only according to the EFS expression and spike row number (Table 1, Fig.
171 1, Supplementary Fig. S2) but also according to other significant ornamental value characters, such as
172 the spike colour at different stages of plant maturation (Supplementary Fig. S3), colour of mature grains
173 (Supplementary Fig. S4), plant height (Supplementary Table S2), spike length and awn/spike ratio
174 (Supplementary Table S3). Spikes with long awns are very attractive (Fig. 1, Supplementary Figs. S3
175 and S5). Spikes with high awn/spike ratio were found among all hybrid combinations of *tw₂* with various
176 *Hd* mutants. However, exclusive lines with respect to the awn/spike ratio were found among *tw₂* × *Lh*,
177 especially *tw₂* × *Br*. Lines exhibiting short spikes but long awns (*tw₂* × *Br* N41, N42 or *tw₂* × *Lh* N29),
178 similar to other barley species, *Hordeum jubatum*, used as ornamental plants, are very attractive (Fig. 1
179 and Supplementary Fig. S2). Exclusive ornamental lines were selected from the progeny of *tw₂* × *Dwh*.
180 However, such imposing lines produce fewer grains and may be propagated only in restricted amounts
181 for special needs. Two lines lack EFS but are awnless, while the parental mutants produce ectopic floral
182 structures or awns (Table 1).

183 The spikes of *tw₂* and most of the single *Hd*-type mutants used in the present study, except *Hcs*,
184 are short, and all *Hd* mutants, except *Lh*, are awnless (Figs. 1 and 2, Supplementary Table S3). Among
185 all cross-combinations, lines were found with a spike length that significantly exceeded that of both
186 parents (Supplementary Table S3). One particularly noteworthy achievement was that lines with EFS on
187 long spikes were found among *tw₂* × *Br* (N38, N40) and *H* × *Br* (N3) (Fig. 1 and Supplementary Fig.
188 S2). These lines also exhibited colour variations (Supplementary Fig. S3). The initial mutants, *Br* and
189 *Dwh*, have especially short spikes and were included in the hybridization for spike length improvement.

190 Plant height as well as straw colour are very important if the straw is used for dry bunches and
191 decorations. Improvements of plant height were obtained for lines composed of *Hd*-type mutants, such
192 as *Lh*, *Mf* and especially *Dwh* and *Br*, with a very short straw. Tall plants with attractive EFS were
193 selected from *tw₂* × *Dwh* (Table 2 and Supplementary Table S2).

194 Straw and spike colour variations were an exclusive peculiarity of lines selected among *tw₂* ×
195 *Br* and *H* × *Br* (Fig. 1 and Supplementary Fig. S3). However, barley grain colour also has a demand in

196 markets, and a large spectrum of grain colour variations was preserved in the collection of ornamental
197 lines, mostly among $tw_2 \times Br$ or $H \times Br$ (Supplementary Fig. S4).

198 New features that were absent in both parents were also observed, which is best illustrated in
199 several lines from $tw_2 \times Lh$. Lines with short awns (N16, N18) or with awns fully transformed into EFS
200 (N9, N21) were selected, while both parents, tw_2 and Lh , have long awns or ectopic structures on long
201 awns (in Lh) (Fig. 1). The new quantitative features might be attributed to lines in which the spike length
202 exceeded approximately twice the spike length of the parental mutants (Supplementary Table S3a), or
203 lines with a high awn/spike ratio that exceeded that of the parental mutants (Supplementary Table S3b).

204 Only an insignificant portion of the lines selected as ornamentals are truly double mutants,
205 expressing phenotypic markers of both Hd and tw mutants – EFS and a tweaked spike. In general, double
206 mutants ($tw_2;Hd$) expressing both Hd and tw phenotypes are genetically unstable and homozygous
207 according to the 305-bp duplication in the $BKn3$ gene (Šiukšta et al. 2015; 2018). Our attention was
208 focused mainly on stable ornamental lines. Regardless, however, phenotypes of individual plants in lines
209 of unstable homeotic double mutants show diversity, and such unstable lines may also have applications
210 for ornamental purposes because of their exotic phenotypes.

211 Selection among the unstable double mutant $tw_2;Hd$ was also performed according to its
212 ornamental value, and the percentage of ornamental lines considering the double mutants $tw_2;Hd$ varied
213 in a wide range among different $tw_2 \times Hd$ cross-combinations (Table 1). The majority of ornamental
214 lines were selected from the $tw_2 \times Br$ cross, but among them, only 5% were characterized as double
215 mutants. The highest number of ornamental lines characterized as double mutants was selected from the
216 $tw_2 \times H$ cross.

217

218 *Quantitative characters of perspective ornamental lines*

219 In Vilnius trials, lines with prospects for wider plant propagation were selected and further studied
220 according to the technology for breeding in the Institute of Agriculture (Dotnuva). These lines were
221 mainly for prospective use for dry bunches and goods (Supplementary Figs. S5 and S6, Supplementary
222 Tables S43 and S5).

223 In all lines tested from 2012–2016, the grain productivity was lower, but to a different extent
224 compared with the reference cultivars. For several lines, $tw_2 \times Br$ N5, N15, $tw_2 \times Lh$ N5, and in many of
225 the $tw_2 \times Dwh$ lines, loss of grain productivity was partially compensated for by small grains, which
226 exhibited a lower weight of 1000 grains (Table 2 and Supplementary Table S4). Resistance to lodging,
227 powdery mildew and net blotch were also variable features, and selection for these traits might be

228 successful. A significant number of lines were even more resistant to net blotch than reference cultivars,
229 but some lines were more susceptible to loose smut (Table 2).

230 Unexpected results were obtained in 2017 (Supplementary Table S5). New ornamental lines were
231 studied, and in an unfavourable year for barley growing, characterized by excessive precipitation in the
232 final period of vegetation (Supplementary Table S1), the three lines, $tw_2 \times Dwh$ N29, N32 and $tw_2 \times Br$
233 N12, were productive (Supplementary Table S5).

234 Under different conditions in 2018, when plants suffered from water deficiency during vegetative
235 growth (Supplementary Table S1), all tested lines were less productive than the reference cultivars.
236 However, lines $tw_2 \times Dwh$ N32 and $tw_2 \times Br$ N12 remained among the most productive ornamental lines
237 in 2018 (Supplementary Table S5).

238 In addition to features important for ornamental line propagation, the protein and starch contents,
239 were studied in selected lines. The starch content was reduced in the studied grains than in the reference
240 cultivars (Table 2, Supplementary Tables S4 and S5). A significant number of lines had protein content
241 in grains exceeding the reference level. However, a negative relationship between productivity and
242 protein content is a well-known phenomenon (Simmonds 1995; Le Bail and Meynard 2003), and most
243 lines studied were less productive (Tables 2 and Supplementary Tables S4 and S5). However, several
244 lines had high protein contents in grains and were relatively productive. Only lines $tw_2 \times Mf$ N3 and tw_2
245 $\times HN4$ were distinguished in the 2012–2016 trials (Tables 2 and S4). The two relatively productive lines,
246 $tw_2 \times Dwh$ N29 and especially $tw_2 \times Dwh$ N32, revealed themselves as prospects for desirable protein
247 content in the 2017 trials (14.5% and 15.1%, respectively; Supplementary Table S5).

248

249 *Molecular characteristics of the selected lines according to the 305-bp duplication in BKn3 gene*

250 An arbitrary 305-bp duplication, serving as specific molecular marker for a mutation in the barley
251 *BKn3* gene, resulting in the *Hooded* phenotype, was studied in three groups of ornamental lines,
252 distinguishing by specificities of expression and inheritance of the main mutant characters of *Hooded*
253 mutations.

254 The first group represents unstable plants that attracted our attention with the development of EFS
255 on relatively long awns on the upper part of the spike but continuously segregating into three phenotypic
256 classes: unstable as a mother plant, stable awnless or with very short awns, and with long awns as in the
257 *WT* or tw_2 (Fig. 2). DNA analysis showed that attractive segregating plants are heterozygous for a 305-
258 bp duplication, i.e., the mutant *BKn3* allele causing the *Hooded* phenotype. The stable awnless plants are

259 homozygous for the mutant allele of the *BKn3* gene. Descendants with the long awns carry the recessive
260 wild allele of the *BKn3* gene lacking the 305-bp duplication (Fig. 3).

261 Homozygous and heterozygous genotypes of segregating lines selected among hybrids of *tw2* with
262 classic *Hooded* mutant in cv. Colseess II differed in the expression of Hooded phenotype (Fig. 2),
263 indicating that the mutant *BKn3* allele, carrying a 305-bp duplication, is not fully dominant in several
264 genetic backgrounds.

265 The second group represents lines selected after hybridization of *tw2* × *Hcs* or *H* × *Hcs* (Fig. 2).
266 Mutant *Hoods on center spikelet* (*Hcs*) shows the weakest *Hooded* phenotype among all *Hd* mutants
267 studied (Fig. 2). It can be assumed that such a phenotype may be caused by the action of the suppressor
268 genes *SuK* (Roig et al. 2004), in which case the plants should preserve a 305-bp duplication. The opposite
269 situation is also possible. The weak *Hooded* phenotype may result from the interaction of *suK* with
270 another barley mutant gene *lks2* despite the presence of the wild allele of the *BKn3* gene (Roig et al.
271 2004). Such a conflicting situation concerning the 305-bp duplication in *tw2* × *Hcs* and *H* × *Hcs* lines
272 required subsequent DNA analysis. Most lines studied were found to be homozygous for a 305-bp
273 duplication. Consequently, an interaction with *suK* is more probable in this case. Only line *tw2* × *Hcs* N1
274 lacks the 305-bp duplication (Fig. 3) but possesses an interesting spike phenotype with very weak awns
275 (Fig. 2).

276 The third group represents the stable ornamental lines. These were selected from different crosses
277 and express EFS and awn development to different extents (Fig. 1). In turn, as shown by DNA analysis,
278 such lines could be divided into two groups, with or without the 305-bp duplication. Regardless, the lines
279 are all homozygous either for the mutant or for the wild allele of the *BKn3* gene (Fig. 3). Among lines
280 carrying the wild allele of the *BKn3* gene, EFS expression was not observed. However, several of the
281 lines have less developed awns (such as *tw2* × *Hcs* N1) or long awns that are soft and imposing (*tw2* ×
282 *MfN6*) (Figs. 1 and 2). It may be proposed that other genes, involved in awn development, are manifested
283 in such cases (Liller et al. 2017).

284

285 **Discussion**

286 Comparison of the two groups of lines, selected as ornamentals after hybridization of either *H* ×
287 *Hd* or *tw2* × *Hd*, showed that more wide and attractive spectra of decorative lines are produced when
288 different types of single homeotic mutants, such as *Hooded* and *tweaky spike2*, are used for hybridization.
289 However, the diversity of ornamental lines has a different nature, resulting not only from the successfully
290 composition of different homeotic mutants.

291 Even the *Hd* mutants itself, which were used in present study and recommended directly as
292 ornamentals, are characterized by significant diversity according to the expression of EFS and other spike
293 characteristics. Their diversity is observed despite the presence of a 305-bp duplication in all *Hd* mutants
294 (Šiukšta et al. 2018, and present study), which is a specific molecular marker for all barley *Hooded*
295 mutants (Müller et al. 1995). Initially, variations among *Hd* mutants must be caused by different genetic
296 background because all *Hd* mutants have different histories of origin (<http://www.ars-grin.gov/npgs/>).
297 However, additional mutations in other sites of the *BKn3* gene may not be excluded. The *Hd* mutants
298 used in the present study were specially selected from the *Hooded* mutant collection as contrasting
299 according to the expression of the Hooded phenotype (Vaitkūnienė et al. 2004), and the diversity of *Hd*
300 mutants in barley collections is much greater. Phenotypic variations among individuals of separate *Hd*
301 mutant lines and even spike phenotype variations within the same plant are naturally observed, due to
302 pleiotropic effects of mutant genes or epigenetic factors, with a temporary character, while all *Hd* mutants
303 and ornamental lines used in the present study have stable distinguishing phenotypes.

304 Genetic background may also be among the basic causes of ornamental line variations in the same
305 hybrid combination due to the combination of two constituents with different genetic backgrounds. The
306 role of genetic background of ornamental line diversity is clear from results following the hybridization
307 of different *Hooded* mutants with the mutant *Hooded* in cv. Colseess II (*H*). Although both parental
308 *Hooded* mutants carried a 305-bp duplication in intron IV of the *BKn3* gene, the new decorative plants
309 were successfully selected from *H* × *Hd* hybrid populations. The most obvious background effect was
310 observed when 6-rowed *H* was hybridized with the 2-rowed anthocyanin-coloured *Hooded* mutant *Brittle*
311 *rachis* (*Br*). Both characters, specific for *Br*, row number (Koppolu et al. 2013; Liller et al. 2015; Youssef
312 et al. 2017) and plant colour (Shoeva et al. 2016; Zhang et al. 2017), are controlled by distinct to *BKn3*
313 genes.

314 Modifier genes also contribute to the diversity of ornamental lines. Their effects on the suppression
315 of the Hooded phenotype has been determined by Roig et al. (2004). The results of 305-bp duplication
316 analysis in the *Hcs* mutant and in respective ornamental lines with *Hcs* suggest that action of modifier
317 genes might occur. Relatively new features, such as short awns or awns that are fully transformed into
318 EFS in *tw2* × *Lh*, despite the fact that *Lh* has EFS on long awns, may be explained by the action of
319 modifier genes in the *tw2* background. Their phenotypes are similar to that caused by the action of the
320 suppressor gene *suK* on the expression of EFS in the *Hooded* mutation (Roig et al. 2004).

321 The hybridization of *Hd* mutants with other homeotic mutant *tweaky spike* significantly broadened
322 to the diversity and success in identifying new ornamental lines, which may be explained the relationship

323 of *tw* mutants to disturbances in the auxin pathway (Šiukšta et al. 2015, 2018). Exotic phenotypes may
324 also be used as ornamentals among descendants of genetic unstable genetic lines, including even those
325 with inflorescence (spike)/flower reversions (Šiukšta et al., 2015), similar to the phenotypes described
326 by Martini and Harlan (1942), Babb and Muehlbauer (2003), and Trevaskis et al. (2007).

327 Our experience shows that various developmental mutants, even those that are not impressive (e.g.
328 mutant *tw*₂), may be successfully used as initial material for the production of ornamental barley lines
329 and cultivars. However, the available large barley genetic collections of developmental mutants
330 (Lundqvist 1992; Druka et al. 2011; Stanca et al. 2013; Dockter et al. 2014; Muñoz-Amatriaín et al. 2014;
331 Terzi et al. 2017 McKim et al. 2018) are under-utilized as a reserve of ornamental forms. The same
332 situation is encountered for collections of other cereals used for ornamental products. Even the barley
333 *Hooded* mutants available in the USDA-ARS National Small Grain Collection
334 (<https://www.ars.usda.gov/>), despite their attractive ectopic floral structures, have appeared in
335 commercial offerings only in recent years, after our previous publication (Siuksta et al., 2012).

336 The hybridization of *tw*₂ × *Hd* allowed not only broadening of the resources of decorative plants,
337 but, more importantly, improvement of plant characters that are important for plant propagation, such as
338 productivity and resistance to lodging or plant diseases. Barley *Hooded* mutants are very attractive by
339 themselves, but improving their spike length, colour, row number, and plant height (if straw is used) is
340 attainable, and wide variation spectra were obtained according to all the abovementioned characters
341 (Tables 1, 2, Supplementary Tables S2–S5).

342 Barley varieties and mutants, used at present or with future prospects for ornamental purposes, may
343 have other attributes that are even more important than the decorative value. Barley Tibetan Purple
344 Hulless, used for its grain colour, is adapted to the severe Tibetan environment and is used as a model
345 plant for stress resistance studies and a source of stress resistance genes (Zeng et al. 2016). It is also used
346 for its unique nutritional value (Chen et al. 2014). The foxtail barley (*H. jubatum*), an aggressive and
347 invasive weed and simultaneously an ornamental plant known for its long attractive awns, is a source for
348 salinity resistance genes (Israelsen et al. 2011). The barley *Hooded* mutants form the basis for breeding
349 of forage cultivars because of the lack of awns and high quality of the resulting forage and hay (Bowman
350 et al. 1994; Hadjichristodoulou 1994; Jacob and Pescatore 2012). Several barley lines selected for
351 ornamental purposes in the present study (as *H* × *Dwh* N6) are also important for their high protein
352 content and increased resistance to net blotch (*Pyrenophora teres*). The high protein content and
353 resistance to net blotch, as well as the susceptibility of several lines to *Ustilago nuda*, are inherited from
354 the *tw*₂ mutant as pleiotropic effects of the *tw* mutation (Rančelis et al. 1994; Mačkinaite et al. 1996).

355 Both initial mutants, *Hooded* and *tweaky spike*, exhibited pleiotropic effects on quantitative characters
356 (Supplementary Tables S2–S5) in comparisons of the respective *Hooded* mutants and mutant *tw* with the
357 reference cultivars (Supplementary Tables S4 and S5) or with cv. Auksiniai II as a Wild Type for mutant
358 *tw₂* (Tables S2 and S3). The shorter plant height among all ornamental lines from $H \times Hd$ or $tw_2 \times Hd$,
359 except $H \times Hcs$ or $tw_2 \times Hcs$ (Supplementary Table S2) or reduced productivity for most of the studied
360 ornamental lines might be also attributed to pleiotropic effects of the *Hd* and *tw* mutations.

361 The common problem associated with many developmental/homeotic mutants, including the
362 *Hooded* mutants, is lower productivity. In the case of *Hooded* mutants, the loss of productivity is
363 associated, at least partially, with awnlessness because of the important role of awns in photosynthesis
364 (Abebe et al. 2009; Jacob and Pescatore 2012). The lower productivity of $H \times Dwh$ N6 and several other
365 lines, valued not only as ornamentals but also for protein content, is compensated for partially by smaller
366 grains per unit volume.

367 In general, ornamental barley and other cereals that are currently used for ornamental purposes
368 form only a small niche in comparison to the crops used for other agricultural purposes, but the
369 enrichment of genetic resources of ornamental cereals must be included in breeding and genetic resource
370 programmes, and single and double homeotic mutants are an important source for enrichment of
371 ornamental plant forms in grasses.

372

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376

377 **Compliance with ethical standards**

378 **Conflict of interest**

379 The authors declare that there are no conflicts of interest.

380

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473

474

475 **Figure captions**

476 **Fig. 1** Spike form diversity of selected ornamental lines and their parental mutants. *Lh* – *Lemma hooded*,
477 *H* – *Hooded* in cv. Colsess II, *Dwh* – *Dense wing hood*, *Mf* – *Multiflorous*, *Br* – *Brittle rachis*, *tw₂* –
478 *tweaky spike₂*.

479

480 **Fig. 2** Several segregating lines (a) and ornamental lines selected among hybrids *tw₂* × *Hcs* and *H* × *Hcs*,
481 (b). The bottom row represents the diversity of morphological spike characters of *wild type* and parental
482 *Hooded* mutants (c). From left: kernel of *wt* with awn and awn transformation into floral structures in
483 mutant *Hooded* in cv. Colsess II (*H*); awn transformation occurring only on central spikelets of mutant
484 *Hoods on center spikelet* (*Hcs*); ectopic floral structures on awns in the mutant *Lemma hooded* (*Lh*) and
485 massive wing-like ectopic structures in the mutant *Dense wing hood* (*Dwh*). *tw₂* – *tweaky spike₂*.

486

487 **Fig. 3** Amplicons containing the 305-bp duplication (649-bp fragment, mutant allele of the *BKn3* gene)
488 or amplicons without the 305-bp duplication (335-bp fragment, wild 305-bp duplication allele of the
489 *BKn3* gene). (I) First group – unstable lines. Homozygous plants have either a 649-bp or 335-bp
490 fragment, heterozygous plants have both fragments. (II) Second group – lines derived from the
491 hybridization *tw₂* × *Hcs* or *H* × *Hcs*. The remaining group represent parental mutants or stable ornamental
492 lines (III group). *tw₂* – *tweaky spike₂*, *H* – *Hooded* in cv. Colsess II, *Dwh* – *Dense wing hood*, *Br* – *Brittle*
493 *rachis*, *Hcs* – *Hoods on center spikelet*, *Lh* – *Lemma hooded*, *Mf* – *Multiflorous*.

494

495 **Supplementary Fig. S1** Diversity of dry ‘palm’ bouquets (in Lithuanian – ‘verbos’) (a-g) and decoration
496 consisting of straws, in Lithuanian named ‘Sodai’ (‘Gardens’) (h).

497

498 **Supplementary Fig. S2** Barley lines named in the section *Common characteristic of ornamental barley*
499 *lines*.

500

501 **Supplementary Fig. S3** Spike colour variations that may be achieved at different spike dormancy stages
502 in lines from *tw₂* × *Br* or *H* × *Br*. AII - cv. Auksiniai II used as *wt*, *tw₂* – *tweaky spike₂*, *H* – *Hooded* in
503 cv. Colsess II, *Br* – *Brittle rachis*.

504

505 **Supplementary Fig. S4** Grain colour and form variations among lines from *H* × *Br* and *tw₂* × *Br*. *wt* –
506 cv. Auksiniai II, *tw₂* – *tweaky spike₂*, *H* – *Hooded* in cv. Colsess II, *Br* – *Brittle rachis*.

507

508 **Supplementary Fig. S5.** Spikes of barley lines tested at the Institute of Agriculture (Dotnuva) from
509 2012–2016. *tw₂* – *tweaky spike₂*, *H* – *Hooded* in Colseess II, *Dwh* – *Dense wing hood*, *Lh* – *Lemma hooded*,
510 *Mf* – *Multiflorous*, *Br* – *Brittle rachis*.

511

512 **Supplementary Fig. S6.** Spikes of barley lines tested at the Institute of Agriculture (Dotnuva) from
513 2017–2018. *H* – *Hooded* in cv. Colseess II, *tw₂* – *tweaky spike₂*, *Lh* – *Lemma hooded*, *Dwh* – *Dense wing*
514 *hood*, *Br* – *Brittle rachis*.