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*Original scientific paper*

# Common wild oat (*Avena fatua* L.) spikelet and seed morphology variation in Bosnia and Herzegovina

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## Abstract

Intraspecific variation in spikelet and seed morphology was assessed for eleven populations of common wild oat (*Avena fatua* L.). Spikelet lengths varied between 1.13 and 1.81 cm, with highly significant differences being recorded between most analyzed populations. Lemma color varied between ocher, light-brown, brown and black, with the majority of analyzed populations (63.64 %) having predominately (≥50%) brown lemma color. Percent of lemma hairiness was also assessed and yielded some highly significant differences between the populations. Length of the awn varied between 2.34 and 4.74 cm, with differences in mean values of awn length per population being statistically significant between several analyzed populations. The angle between the awn and the dorsal surface of lemma was also studied and varied between 85.76° and 140.71°. Analysis of grain length has shown highly significant differences only between the populations from Bačevci (BIH02) and Aleksandrovac (BIH03), when compared with other nine analyzed wild oat populations, while contrary to this, grain mass was shown to be a highly significant factor when comparing wild oat populations. Consequently, it can be theorized that the wide range of variation documented for certain seed morphology traits results from a high local differentiation potential of wild oat populations.

*Key words:* genus *Avena*, wild oat, *Avena fatua*, morphological parameters, morphological variability

## Introduction

Weeds present an important barrier in achieving high yields in agricultural production. The genus *Avena* is comprised of 30 species (Loskutov and Rines, 2011; Fu et al., 2019), displaying a high morphological and ecological diversity across their distribution range (Liu et al., 2017). *Avena fatua* L. is considered to be among the worst annual weeds in cereals in the temperate zone (Li et al. 2007), causing greater problems in agriculture, when compared to its closely related congener *Avena sterilis* subsp. *ludoviciana* (Durieu) Gillet & Magne (Bajwa et al., 2017; Jäck et al., 2017). Although the genus *Avena* has most likely originated in the western part of the Mediterranean region (Loskutov, 2008), the hexaploid group, including *Avena fatua* and common oat (*Avena sativa* L.), is thought to have formed in central Asia, within the centre of crop origin (Thurston and Phillipson, 1976; Loskutov, 2008).

*Avena fatua* is an annual grass, up to 90 (120) cm high. Its stems are either solitary or tufted, with linear, hairless leaves. The inflorescence is a loose panicle and the fruit is an oblong spikelet (Botha, 2001; DiTomaso and Healy, 2007). It is a wind-pollinated species, propagated by seeds (DiTomaso and Healy, 2007) and one plant (with 20 tufted stems), growing in favorable conditions, can produce up to 1500 seeds (Morrow and Gealy, 1982). The optimal temperature of wild oat germination is 15-18 $^{0}$C (Božić and Stevanović, 2012; Saulić et al., 2015) and its seeds can maintain their viability in the soil for up to 10 years (DiTomaso and Healy, 2007).

Bearing in mind the high morphological and ecological variability characteristic for the entire genus *Avena*, the aim of this study was to evaluate the morphological variability of wild oat spikelets and seeds in the territory of Bosnia and Herzegovina.

## Material and Methods

Wild oat seeds were collected from 11 populations of wild oat in wheat and rye fields at the beginning of summer in 2015 in Bosnia and Herzegovina. The populations were separated by at least 25 km, and the location of each sampling site was recorded using a hand-held Garmin GPS device.

Information on the sampling sites is given in Table 1. A total of 330 samples (30 samples per population) were analyzed.

Table 1. Sampling sites of the selected wild oat populations

| Population name | Locality | Latitude | Longitude | Altitude (m) |
|---|---|---|---|---|
| BIH01 | Bijeljina | +44.770327 | +19.234344 | 88 |
| BIH02 | Bačevci | +44.090345 | +19.507983 | 204 |
| BIH03 | Aleksandrovac | +44.9662778 | +17.325666 | 107 |
| BIH04 | Patkovača | +44.731119 | +19.22426 | 94 |
| BIH05 | Priboj | +44.603235 | +18.942976 | 241 |
| BIH06 | Glavičica | +44.600266 | +19.176308 | 150 |
| BIH07 | Ugljevička obrežina | +44.696203 | +19.036466 | 163 |
| BIH08 | Ročević | +44.534486 | +19.147164 | 123 |
| BIH09 | Čelopek | +44.44049 | +19.136439 | 131 |
| BIH10 | Banjaluka | +44.78838 | +17.20330 | 154 |
| BIH11 | Hrvaćani | +44.85787 | +17.45322 | 185 |

Based on relevant morphological parameters, all collected wild oat samples were determined to belong to *Avena fatua*. A total of eight morphological features were chosen for this study: eight quantitative and one qualitative. Wild oat spikelets were photographed on a stereo trinocular microscope (Micro-SC2 EUinstruments). The measurements of spikelet length and width, awn length and the angle between the awn and the dorsal lemma surface and grain length were done subsequently using ImageJ software (Abramoff et al. 2004). Grain mass was also measured using an Analytical Balance (CANBEC120, COLO LabExperts). Lemma hairiness was assessed visually and graded on a scale of 0-100% and lemma color was assessed and assigned to each sample following a scale: white - golden - yellow - ocher - crème - copper – light brown - brown - black. Variability of the assessed morphological traits was analyzed using basic statistical analysis (Descriptive statistics), one-way analysis of variance (ANOVA) and a t-test for the comparison between means of the selected parameters in the studied populations in Statistica 7.0 (StatSoft Inc., Tulsa, USA).

## Results and Discussion

Eight morphological traits of wild oat spikelets and seeds were analyzed on a total of 330 samples, from eleven populations. Results of descriptive statistics point to a low to moderate level of variation in these traits within the analyzed populations, which is evident in the Box and Whisker plot diagrams (Figures 1, 2 and 3). Analysis of variance (ANOVA) was used to test the significance of differences in mean values for all the studied morphological parameters (Table 2). Given that p values were below the threshold for extreme statistical significance (p

< 0.001) for all the chosen morphological traits, they can be considered as valuable in the study of morphological variability of the selected wild oat populations.

Table 2. Results of ANOVA analysis of mean values of eight selected morphological traits between 11 populations of wild oat in Bosnia and Herzegovina

| Character name and unit | N | F | p |
|---|---|---|---|
| Spikelet length (cm) | 11 | 27.72575 | 0.000000** |
| Spikelet width (cm) | 11 | 7.55608 | 0.000000** |
| Color of the lemma | 11 | 14.14534 | 0.000000** |
| Lemma hairiness (%) | 11 | 26.54991 | 0.000000** |
| Awn length (cm) | 11 | 8.66624 | 0.000000** |
| Angle of the awn (°) | 11 | 12.24237 | 0.000000** |
| Grain length (mm) | 11 | 23.67234 | 0.000000** |
| Grain mass (g) | 11 | 12.09875 | 0.000000** |

N – number of analyzed populations; F – Fisher's coefficient;
p – level of significance

Length of wild oat spikelets in the total sample of 330 spikelets from 11 populations varied between 1.13 cm as its minimum value and 3.38 cm as its maximum value, with population BIH09, from Čelopek (BIH09), having the lowest mean value (1.53 ± 0.17 cm) and BIH02 (Bačevci) the highest mean value at 2.07 ± 0.22 cm (Figure 1). The spikelet width varied between 0.12 and 0.57 cm. As expected, the populations from Čelopek and Bačevci were characterized by the lowest (0.20 ± 0.02 cm) and highest (0.26 ± 0.02 cm) mean values of spikelet width, respectively, which is in line with their mean spikelet length values.

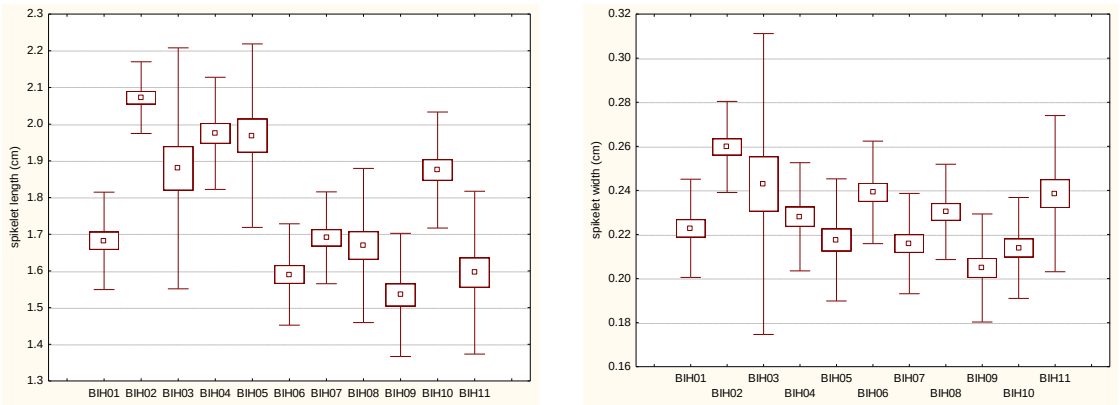


Figure 1. Box and Whisker plot of basic statistical parameters of the total spikelet length (a) and width (b) trait in wild oat populations from Bosnia and Herzegovina (middle point – Mean value; box – Mean ± SE; whisker – Mean ± SD)

The differences in mean spikelet length between most analyzed populations were highly significant (Table 3). Contrary to this, mean spikelet width proved to be a less variable morphological character between the selected populations, being highly significant ($p \leq 0.01$) in 40% cases (Table 3).

Based on spikelet length, populations Bačevci (BIH02), Patkovača (BIH04) and Priboj (BIH05) can be highlighted, as extreme statistical significance ($p<0.001$) is recorded in the differences of mean values between these three and a number of other studied populations (BIH02 / BIH06-11; BIH04 and BIH05 / BIH06-09 and BIH11, Table 3).

Table 3. T-test comparing the means of total spikelet length (white fields) and width (grey fields) between the studied wild oat populations in Bosnia and Herzegovina

| | BIH01 | BIH02 | BIH03 | BIH04 | BIH05 | BIH06 | BIH07 | BIH08 | BIH09 | BIH10 | BIH11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BIH01 | 1 | 0.000000** | ns | ns | ns | 0.007484** | ns | ns | 0.004247** | ns | 0.043990* |
| BIH02 | 0.000000** | 1 | ns | 0.000001** | 0.000000** | 0.000595** | 0.000000** | 0.000001** | 0.000000** | 0.000000** | 0.006358** |
| BIH03 | 0.003384** | 0.003165** | 1 | ns | ns | ns | 0.044112* | ns | 0.005602** | 0.031333* | ns |
| BIH04 | 0.000000** | 0.004734** | ns | 1 | ns | ns | ns | ns | 0.000531** | 0.024652* | ns |
| BIH05 | 0.000001** | 0.038779* | ns | ns | 1 | 0.001837** | ns | ns | ns | ns | 0.013177* |
| BIH06 | 0.011184* | 0.000000** | 0.00004** | 0.00000** | 0.00000** | 1 | 0.000245** | ns | 0.000001** | 0.000084** | ns |
| BIH07 | ns | 0.000000** | 0.00457** | 0.00000** | 0.00000** | 0.00473** | 1 | 0.015060* | ns | ns | 0.004605** |
| BIH08 | ns | 0.000000** | 0.004514** | 0.00000** | 0.00001** | ns | ns | 1 | 0.000076** | 0.006202** | ns |
| BIH09 | 0.000384** | 0.000000** | 0.000004** | 0.00000** | 0.00000** | ns | 0.000143** | 0.008091** | 1 | ns | 0.000069** |
| BIH10 | 0.000004** | 0.000000** | ns | 0.015651* | ns | 0.00000** | 0.000005** | 0.00007** | 0.00000** | 1 | 0.002238** |
| BIH11 | ns | 0.000000** | 0.00023** | 0.00000** | 0.00000** | ns | 0.045687* | ns | ns | 0.00000** | 1 |

**- highly statistically significant ($p < 0.01$), * statistically significant ($p < 0.05$); ns- not significant

Lemma color was assessed, with crème and copper being recorded in only one population each (Glavičica and Banjaluka, respectively). Three populations – Patkovača (BIH04), Priboj (BIH05) and Ročević (BIH08), had ocher lemma color, in 3.33%, 20% and 3.33% of samples, respectively. Light brown, brown and black lemma colors were most frequent among the samples, recorded in eight, ten and seven populations, respectively. Light brown color of lemma was documented in eight populations, with the highest proportion of samples of this color being characteristic for populations from Bijeljina (BIH01) (46.67%) and Patkovača (BIH04) (50%). Brown lemma color was recorded in a majority of analyzed populations (63.64%), where spikelets are predominately (≥50%) of this color. Black color of lemma was recorded with the highest value (96.67%) in the population BIH02, from Bačevci.

Lemma hairiness was also assessed and varied between 0 and 95% in some populations. Population from Bačevci had the lowest mean hairiness percentage ($8 \pm 7\%$), while BIH03 was

characterized by the highest mean hairiness percentage: 60 ± 21%. However, high values of standard deviation (Figure 2) in all the analyzed populations point to the fact that this morphological trait has proven to also be variable within the same population, therefore making it less informative in the study of interpopulation variation of the studied wild oat populations.

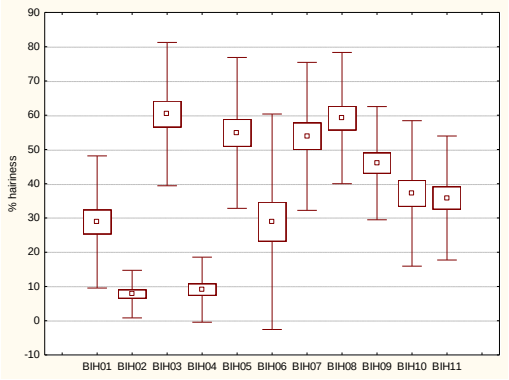


Figure 2. Box and Whisker plot of basic statistical parameters of lemma hairiness (in %) in wild oat populations from Bosnia and Herzegovina (middle point – Mean value; box – Mean ± SE; whisker – Mean ± SD)

Length and angle of the awn were also studied. Length of the awn varied between 2.34 and 4.74 cm, while the angle of the awn and the dorsal lemma surface varied between 85.76° and 140.71°. The lowest mean values of awn length (3.15 ± 0.39 cm) and the angle of the awn (98.87 ± 5.41°) were recorded for wild oat population from Čelopek (BIH09). On the other hand, the highest mean value of awn length (3.82 ± 0.50 cm) was characteristic for the population from Priboj (BIH05) and awn angle (118.2 ± 10.13°) in the population from Bijeljina (BIH01).

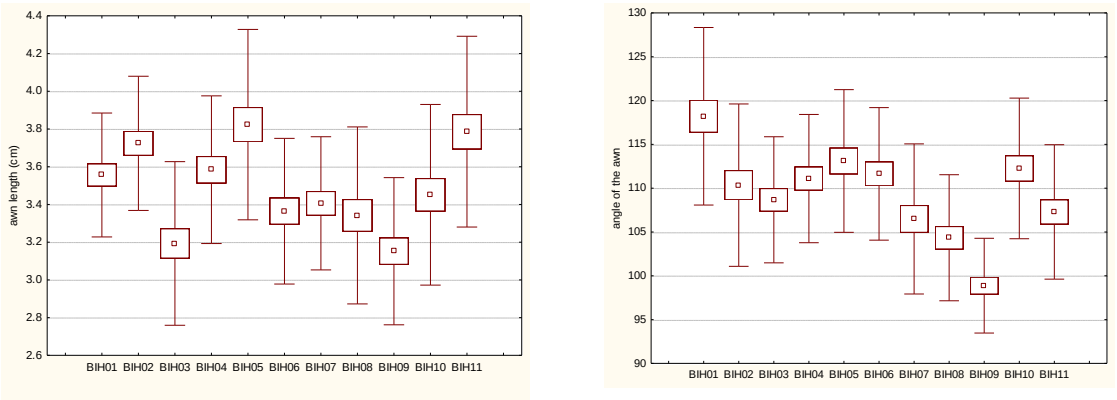


Figure 3. Box and Whisker plot of basic statistical parameters of awn length (a) and angle of the awn (b) in wild oat populations from Bosnia and Herzegovina (middle point – Mean value; box – Mean ± SE; whisker – Mean ± SD)

Statistical significance of the interpopulation differences for these two morphological traits are shown in Table 4, where it can be observed that both morphological traits act as strong parameters of interpopulation variation in wild oat. Based on statistical analysis of the awn length parameter it has been shown that highly significant differences are present between population BIH02 and populations BIH06-09, population BIH05 and BIH11 and populations BIH06-10 (Table 4).

Meanwhile, statistical analysis based on the angle between the awn and the dorsal lemma surface has shown that the most significant differences are present between populations BIH01, BIH08 and BIH09 and the remaining eight wild oat populations (Table 4).

Table 4. T-test comparing the means of awn length (white fields) and angle between the awn and the dorsal lemma surface (grey fields) of the studied wild oat populations in Bosnia and Herzegovina

| | BIH01 | BIH02 | BIH03 | BIH04 | BIH05 | BIH06 | BIH07 | BIH08 | BIH09 | BIH10 | BIH11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BIH01 | 1 | 0.002700** | 0.000093** | 0.002857** | 0.035769* | 0.006069** | 0.000010** | 0.000000** | 0.000000** | 0.014480* | 0.000016** |
| BIH02 | ns | 1 | ns | ns | ns | ns | ns | 0.006878** | 0.000000** | ns | ns |
| BIH03 | 0.000557** | 0.000003** | 1 | ns | 0.029353* | ns | ns | 0.023285* | 0.000000** | ns | ns |
| BIH04 | ns | ns | 0.000538** | 1 | ns | ns | 0.029072* | 0.000655** | 0.000000** | ns | ns |
| BIH05 | 0.018301* | ns | 0.000003** | 0.044993* | 1 | ns | 0.003301** | 0.000044** | 0.000000** | ns | 0.006117** |
| BIH06 | 0.042516* | 0.000407** | ns | 0.032484* | 0.000210** | 1 | 0.016772* | 0.000325** | 0.000000** | ns | 0.031434* |
| BIH07 | ns | 0.000973** | 0.041681* | ns | 0.000465** | ns | 1 | ns | 0.000122** | 0.009325** | ns |
| BIH08 | 0.044972* | 0.000764** | ns | 0.033990* | 0.000322** | ns | ns | 1 | 0.001520** | 0.000170** | ns |
| BIH09 | 0.000057** | 0.000000** | ns | 0.000070 ** | 0.000000** | 0.038416* | 0.010520* | ns | 1 | 0.000000** | 0.000008** |
| BIH10 | ns | 0.015155* | 0.032805* | ns | 0.004868** | ns | ns | ns | 0.010256* | 1 | 0.017399* |
| BIH11 | 0.041387* | ns | 0.000009** | ns | ns | 0.000601** | 0.001322** | 0.000834** | 0.000001** | 0.010868* | 1 |

**- highly statistically significant ($p < 0.01$), * statistically significant ($p < 0.05$); ns- not significant

Grain length and mass of the collected samples were also analyzed. The length of grain varied between 5 and 11 mm, with population from Priboj (BIH05) having the lowest average grain length (6.53 ± 0.90 mm) and population from Bačevci (BIH02) having the highest average grain length (8.93 ± 0.61 mm).

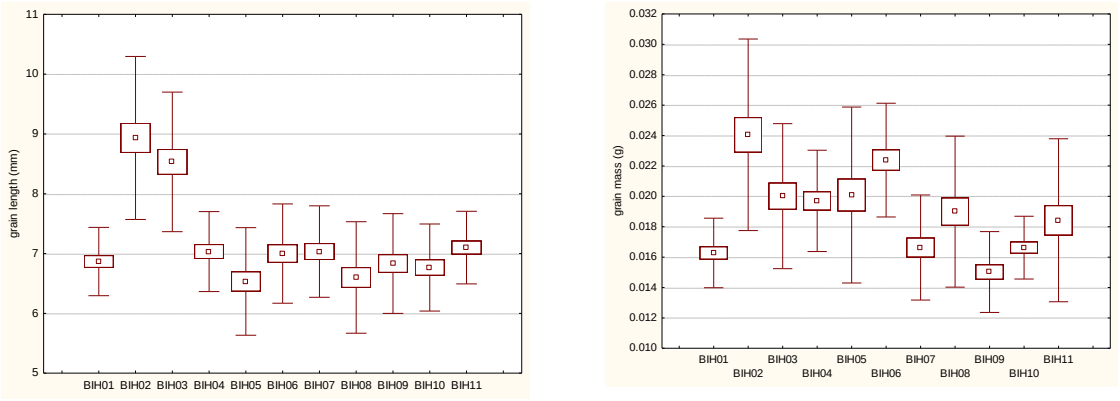


Figure 4. Box and Whisker plot of basic statistical parameters of grain length (a) and width (b) in wild oat populations from Bosnia and Herzegovina (middle point – Mean value; box – Mean ± SE; whisker – Mean ± SD)

Analysis of grain length has shown extremely significant differences (p = 0.00000) only between the populations from Bačevci (BIH02) and Aleksandrovac (BIH03), when compared with other nine analyzed wild oat populations, while contrary to this, grain mass was shown to be a highly significant factor in 55% of cases, when comparing the analyzed wild oat populations (Table 5). Grain mass varied between 0.001 and 0.037 g (data not shown), which is in line with the morphological characteristics of *A. fatua* in the literature. According to Фисюнов (1984) grain size in *A. fatua* is expected to be 8-16 x 1,75-2,50 x 1,25-2,25 mm, with an average mass of 1000 seed of 15-25 g.

Table 5. T-test comparing the means of grain length (white fields) and grain weight (grey fields) between the studied wild oat populations in Bosnia and Herzegovina

| | BIH01 | BIH02 | BIH03 | BIH04 | BIH05 | BIH06 | BIH07 | BIH08 | BIH09 | BIH10 | BIH11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BIH01 | 1 | 0.000000** | 0.000281** | 0.000020** | 0.001400** | 0.000000** | ns | 0.008517** | ns | ns | 0.048077* |
| BIH02 | 0.000000** | 1 | 0.006938** | 0.001461** | 0.013885* | ns | 0.000001** | 0.001046** | 0.000000** | 0.000000** | 0.000445** |
| BIH03 | 0.000000** | ns | 1 | ns | ns | 0.036278* | 0.002644** | ns | 0.000006** | 0.000730** | ns |
| BIH04 | ns | 0.000000** | 0.000000** | 1 | ns | 0.004818** | 0.000900** | ns | 0.000000** | 0.000067** | ns |
| BIH05 | ns | 0.000000** | 0.000000** | 0.017618* | 1 | ns | 0.006775** | ns | 0.000054** | 0.003098** | ns |
| BIH06 | ns | 0.000000** | 0.000000** | ns | 0.041218* | 1 | 0.000000** | 0.004146** | 0.000000** | 0.000000** | 0.001578** |
| BIH07 | ns | 0.000000** | 0.000000** | ns | 0.023921* | ns | 1 | 0.036690* | 0.047862* | ns | ns |
| BIH08 | ns | 0.000000** | 0.000000** | 0.043022* | ns | ns | ns | 1 | 0.000285** | 0.019006* | ns |
| BIH09 | ns | 0.000000** | 0.000000** | ns | ns | ns | ns | ns | 1 | 0.011558* | 0.002867** |
| BIH10 | ns | 0.000000** | 0.000000** | ns | ns | ns | ns | ns | ns | 1 | ns |
| BIH11 | ns | 0.000000** | 0.000000** | ns | 0.005888** | ns | ns | 0.016835** | ns | ns | 1 |

**- highly statistically significant (p < 0.01), * statistically significant (p < 0.05); ns- not significant

In general, high interpopulation morphological variation of the studied populations of wild oat, despite their relatively close proximity, can be explained by the fact that morphological variability is highly characteristic for the representatives of this genus. Furthermore, *A. fatua* is a widely distributed weed species, which can adapt to different soil types and environmental conditions (e.g. soil pH below 4,5, Holm et al., 1977; on alkaline soils, Korniak, 1996; Korniak et al., 2000), and therefore it is expected to exhibit high variations in morphology.

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