

Variability of selected traits in meadow fescue (*Festuca pratensis* Huds.) plants with different susceptibility to seed shattering[†]

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Abstract: In meadow fescue (*Festuca pratensis* Huds.), a perennial fodder grass plant characterized by high quality and yield potential, seed shattering makes seed production difficult. The objective of the research was to compare two subpopulations, differing in a tendency to seed shattering, in respect of variability in the beginning of the heading and flowering stages, morphological traits (plant height and panicle length), and changes occurring at the site of the bonding between the caryopsis and the pedicel. The subpopulations were obtained by divergent selection carried out on four meadow fescue cultivars. Significant variability in years and subpopulations in respect of the tested traits was found. The subpopulation with a low tendency to seed shattering was characterized by a 2-3-day delay in the beginning of the heading and flowering stages. However, in both the subpopulations good flowering stage synchronization was found, its variability being several times smaller compared with the heading stage. Between the starting dates of these stages a significant correlation was found ($r=0.828$; $p<0.01$). The clones with a high tendency to seed shattering were approximately 7 cm shorter, while the length of panicles was similar in both the subpopulations. The analysis with the use of a scanning microscope showed that in genotypes with a low tendency to shattering the formation of the abscission layer was four days late, which may confirm the effectiveness of the carried out selection.

Keywords: abscission; *Festuca pratensis*; flowering; heading; morphological traits; seed shattering

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1. Introduction

Meadow fescue (*Festuca pratensis* Huds.) is a perennial fodder grass plant characterized by high quality and yield potential. Owing to its high frost resistance it is commonly grown in North-West and East-Central Europe [1]. In addition, its fodder value is higher compared with other perennial grasses [2]. In meadow fescue seed production the main problem consists in heavy seed shattering which already begins in partially green panicles. In that species the occurrence of one caryopsis in a spikelet is enough to induce the formation of the abscission layer which causes the separation of the caryopsis from the pedicel and its falling out of the spikelet [3]. Early harvest, aimed at limiting seed losses, may result in the loss of late-ripening genotypes.

The breeding works aimed at decreasing the tendency to seed shattering in meadow fescue cover both morphological traits [4] and the panicle structure, as well as the strength of the caryopsis bonding in spikelets [5]. However, as the available literature shows, the site at which the abscission layer between the caryopsis and the spikelet pedicel is formed in meadow fescue has not been examined using scanning microscopy. In grass breeding, one of the factors which make the selection difficult is a considerable genotype-environment interaction which in individual species contributes to high intra-cultivar variability

[6]. Habitat conditions affect not only the heading and flowering phenophases, mainly through air temperature and amount of precipitation [7], but also morphological traits of plants. As the results of many studies show, the amount of seeds produced in grasses depends directly on the number of generative shoots and their length [4,8-9], as well as on the length of panicles [6,10].

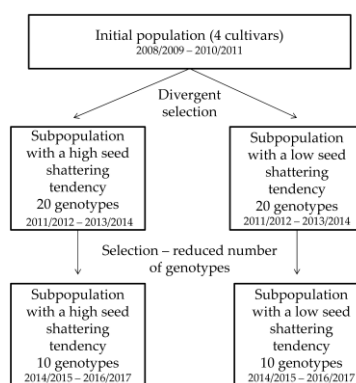
The objective of the research was to compare two subpopulations of meadow fescue obtained via divergent selection, differing in the tendency to seed shattering, in respect of the variability of the heading and flowering stages, morphological traits, such as plant height and panicle length, and to use scanning electron microscopy to evaluate the changes undergoing at the site of the bonding between the caryopsis and the spikelet pedicel which result in seed shattering.

2. Material and Methods

2.1. Plant material and experimental design

Field experiments were carried out in the years 2012-2017 at the Experimental Station of the University of Agriculture in Prusy near Krakow, Poland (N 50°07'03" and E 20°05'13"). The initial material for performing the divergent selection for low and high seed shattering tendency included meadow fescue (*Festuca pratensis* Huds.) cultivars: Cykada, Skawa, Skiba and Skra (breeder: Małopolska Hodowla Roślin HBP Sp. z o.o., Poland).

The divergent selection was performed on the basis of the results of the experiment carried out in the years 2008-2011 (Scheme I). The susceptibility to seed shattering, determined under laboratory conditions with the use of a modified laboratory shaker, was the main criterion for this selection [11]. The plants in which seed losses exceeded 20% were defined as the plants susceptible to seed shattering, while plants in which seed shattering was lower (losses <20%) were ranked among the group of plants with a low seed shattering tendency. Selected plants were vegetatively propagated; as a result, five plants within each clone were obtained. The clones were planted, maintaining spatial isolation, according to the randomized block design with five replications, five plants each. Over the entire research period, in accordance with the OECD methodology [12], the following observations were performed: starting dates of the heading and flowering stages were determined, and one week after the beginning of the flowering stage plant height and panicle length measurements were taken.



Schema I. Outline of the selection for a varied seed shattering tendency carried out on meadow fescue plants.

2.2. Scanning electron microscopy (SEM)

After the divergent selection the site of the bonding between the caryopsis and the spikelet pedicel was observed using scanning microscopy. Starting with the 18th day after the beginning of the flowering stage, at four dates (from the 14th till the 25th of June 2012), from the second branch of panicles samples of spikelets were taken. In both the subpopulations, because of different heading and flowering dates, panicles characterized by the

identical degree of ripeness, similar plant habit, length and number of branches were selected. Samples of spikelets were soaked with 2.5% glutaraldehyde in 0.1 M PBS (phosphate-buffered saline, pH 7.2) for 15 minutes and then postfixed with 1% osmium tetroxide in PBS for 4 h at room temperature. They were dehydrated using an ethanol series (30%, 50%, 70%, 80%, 90%, 95%, 100%), than dried with liquid CO₂ in a critical point dryer (Type E3100 Industrial LADD, USA). In the next step samples were coated with gold using a sputter coater (JEOL JFC-1100E, Japan). Finally, samples were observed using a scanning electron microscope (JEOL JSM 5410, Japan).

2.3. Statistical analysis

The results were analyzed in respect of variability, calculating the mean values and the coefficient of variation (CV%). The effects of years and the differentiation in the selected genotypes in respect of the seed shattering tendency on the heading and flowering dates as well as on plant height and panicle length were determined on the basis of two-way ANOVA. The tests were performed according to a mixed model, in which years were the random factor and the genotypes were the fixed factor. The mean values were compared using Tukey’s HSD test at p<0.05. The correlation between heading and flowering starting dates and the length of shoots and panicles was determined using Pearson’s correlation coefficient at p<0.05. The analyses were performed with the use of Statistica 13 [13].

3. Results and Discussion

3.1. Characterization of the obtained subpopulations

During the research the growing conditions and plantation age significantly affected the course of the phenological phases. Before heading and while entering the heading and flowering stages the mean air temperature fluctuations were about 2 °C (Table 2). The amount of precipitation underwent much greater changes; its maximum values were noted in April 2017, May 2014 and 2015, and in June 2012 and 2013.

In the years 2012 and 2013 heading began at the latest date, i.e. between the 20th and 22nd of May, and in 2012, 2015 and 2017 flowering did not occur until 3rd- 6th of June (Figure 1 a, b). In the first years after selection, i.e. in 2012 and 2015, in plants showing a low seed shattering tendency the heading stage was significantly late. Only in 2015 the delay was found also for the beginning of the flowering stage. In other years flowering in both the subpopulations occurred at a similar date. In the years 2012, 2015 and 2017 in both the subpopulations the number of days between the two tested phonological phases was the highest (approximately 15 days). A significant correlation between the heading and flowering dates (r=0.828; p<0.01; n=100) was found.

Table 2. Weather conditions during the heading and flowering, as well as seed ripening stages observed at the Experimental Station in Prusy in the years 2009-2017

Temperature [°C]	Precipitation [mm]

The range of variability for the observed stages was strongly affected by years. In the third growing year (2014) both the populations showed over twice as high variability in the earliness of heading compared with other growing seasons (Figure 1a). The best synchronization of the beginning of the flowering stage was found in the year 2012 (Figure 1b). In grass growing, it is important that the successive development stages should occur synchronically during the entire course of production. In fescues a delay in the beginning of the heading stage is usually connected with decreased seed yield [14].

The mean length of shoots was 116 cm over the entire research period. In the successive years of growing, significantly higher, by 7 cm on average, plants occurred in the population of genotypes with a low seed shattering tendency (Figure 1c). In both the subpopulations a tendency to increasing the plant height in the second year of growing was observed. The highest variability was found for the genotypes with low susceptibility to seed shattering in the first year after cloning the plants, both after the first and the second selection (Figure 1c). In other years of research variability in both the populations was much the same, i.e. 7%.

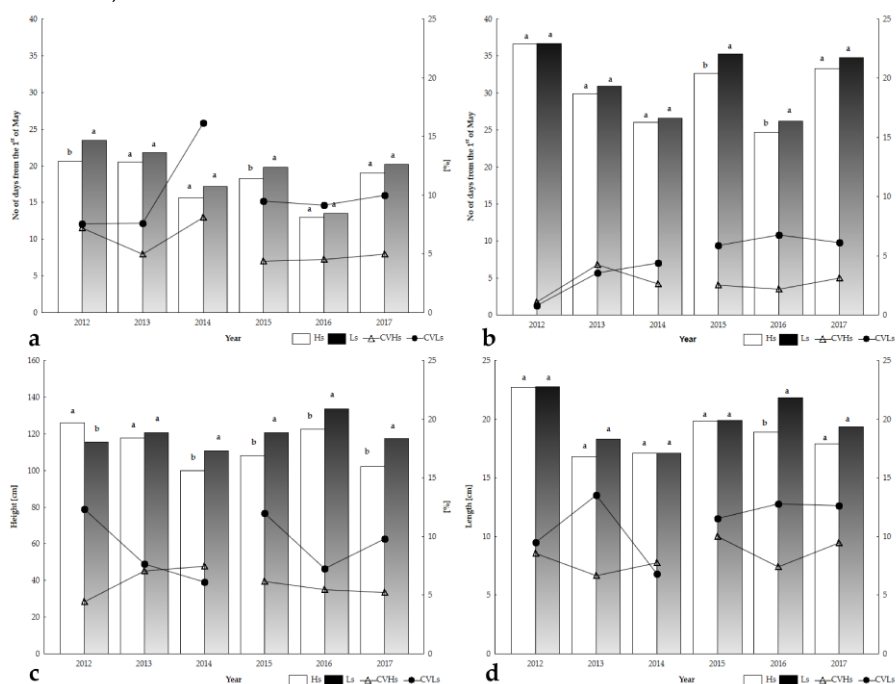


Figure 1. Variability of the starting dates of the heading (a) and flowering (b) stages, as well as plant height (c) and panicle length (d) in meadow fescue clones differing in the seed shattering tendency (Hs – high shattering; Ls – low shattering; CV – coefficient of variation; the bars marked with the same letter in a given growing year present the means which do not differ significantly according to Tukey’s HSD test)

Changes in the length of shoots were accompanied by changes in the length of panicles (Figure 1d). In the population with high susceptibility to seed shattering the correlation between these two traits was higher ($r=0.582$; $p<0.01$; $n=100$) as compared with the population with low susceptibility to seed shattering ($r=0.481$; $p<0.01$; $n=100$). As compared with the first year of analyzing the plants obtained after the divergent selection (2012), in which the length of panicles in both the populations was 22 cm, the decrease in that trait in successive years and after the second selection was 1 cm to 6 cm (Figure 1d). Genotypes with lower susceptibility to seed shattering were characterized by almost double variability of panicle length, ranging from 9% to 13% (Figure 1d). In meadow fescue growing, panicle length is of great importance because, as Mäkelä and Kousa [15] demonstrated, half of fertile spikelets may be located on six or seven branches in the bottom part of the panicle, while sterile spikelets are accumulated mainly in the middle part of the panicle.

3.2. Analysis of the site of the bonding between the caryopsis and the spikelet pedicel

In a majority of plants the formation of the abscission layer between a fruit or a seed and the maternal plant is essential for spreading seeds [16]. In part of plants of the grass family spikelets may break down, or in whole single-seeded or multi-seeded spikelets seed shattering may occur. Observations of the ventral and dorsal sides of meadow fescue caryopses, performed with the use of SEM, revealed the separation process occurring between the tissues at the base of the caryopsis and the spikelet tissues. The photos of the dorsal side, taken both at the first and successive sampling dates, present a more advanced caryopsis separation process (Figure 2 a-d, Ld and Hd).

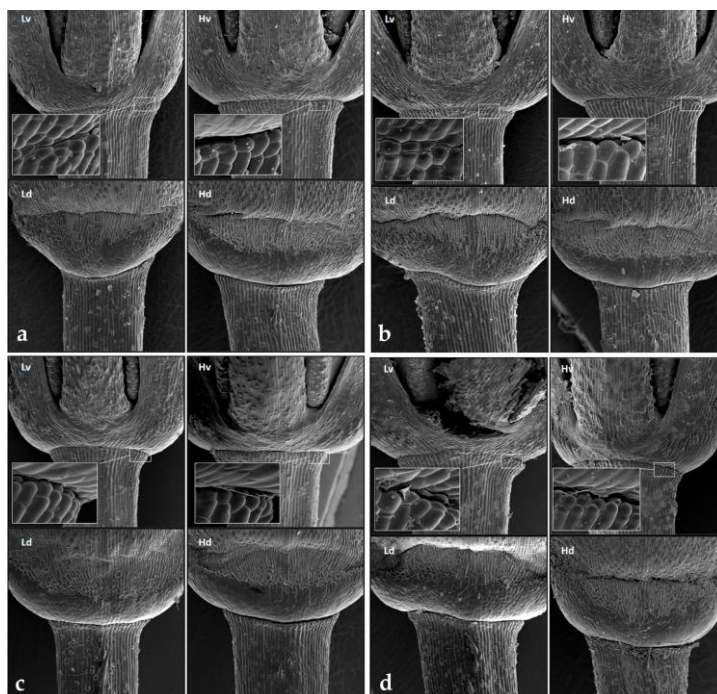


Figure 2. Formation of the abscission layer between the caryopsis and the spikelet at successive sampling dates (a-d). The genotype with a low seed shattering tendency: Lv - as seen from the ventral side, Ld – as seen from the dorsal side; the genotype with a high seed shattering tendency: Lv – as seen from the ventral side, Ld – as seen from the dorsal side; 150x magnification; magnification of the fragment - 1500x

At the ventral side, the crack that is formed at the base of the caryopsis at the first sampling date was distinctly smaller in the genotype with a low seed shattering tendency

(Figure 2 a, Lv). At the successive sampling dates the crack in that genotype did not reach the caryopsis axis (Figure 2 b-d, Lv). In the genotype with a high tendency to seed shattering the formation of the abscission layer proceeded faster. Already at the second sampling date the crack occurred along the whole width of the caryopsis base (Figure 2 b-d, Hv). Goliński [5] showed that in meadow fescue, before the caryopses reach the wax ripeness stage, the force needed to tear the spikelet away from the pedicel is relatively high. However, at the stage of full ripeness the spikelet could be separated using minimal force.

4. Conclusions

The selection which resulted in obtaining the tested subpopulations with different susceptibility to seed shattering was based on the simulation of the conditions prevailing on a plantation (panicles hit one another under the influence of wind or seed shattering occurs during mechanical harvest). Thus, the results of the observations with the use of a scanning microscope may indicate the effectiveness of the performed selection, in spite of the fact that the fragility of the caryopsis-spikelet bonding made it difficult to examine the caryopsis separation process occurring till the sampling date.

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