

VARIABILITY OF MEADOW FESCUE ACCESSIONS AND PRODUCTIVITY AND QUALITY OF THEIR POLYCROSS PROGENIES

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ABSTRACT

The collection investigated included nineteen meadow fescue accessions, 16 wild populations, and 3 commercial cultivars. The first part of the experiment was performed in a plant nursery at a distance plant of plant 60x60 cm. The experiment was done as a randomized block design with 30 plants per accession. This part of the experiment aimed to determine values and variability for the studied traits (heading date, plant height, length of leaf and the number of generative and vegetative tillers per plant and dry matter yield per plant) in two productive years. Data processing was done by ANOVA analyses. The obtained results show that the studied accessions had significant within and among variability for all parameters. The number of generative tillers per plant, plant height and leaf length positively affected dry matter yield per plant. The highest dry matter yield per plant was observed for accessions: FP 1 and FP 4, but the best ratio between dry matter yield and number of vegetative tillers per plant, traits that influenced dry matter quality, was scored for accessions FP 8, FP 7, FP 3, FP 10 and FP 16. The second part of the trial investigated the productivity and biomass quality of the best 26 progenies selected from the polycross field and formed from the plant with the best performance from the nursery. They are tested in plots 5x2 m, as a randomized block design, in 3 replications. The most productive progenies with high biomass quality originated from accession FP 8, FP 3 and FP 13.

Keywords: meadow fescue, accessions, variability, productivity, biomass quality.

INTRODUCTION

Meadow fescue (*Festuca pratensis* Huds.) is important perennial grass species, well adapted to cold climates. This species can grow on wet and cold soils, even on flooded during the winter. Also, meadow fescue has good drought resistance during the growing season. It is a very productive species with high nutritional value and digestibility of biomass. Meadow fescue is indifferent to the management regime but very rare in intensively grazed grasslands. It is mainly grown for hay and silage production in mixtures with many grasses and legumes. This species provides a consistent balance of yield, quality, palatability and persistence.

Meadow fescue ($2n=2x=14$) is a diploid outbreeding grass species with expressed self-incompatibility. Belongs to the genus *Festuca* which contains about 600 species (Cheng et al., 2016). The presence of high

self-incompatibility and inbreeding depression complicates the breeding process and makes breeding focus on the creation of synthetic cultivars or improved heterogeneous populations which consist of genetically uniform individuals who have many similar characteristics. The concept of breeding and creating cultivar of outbreeding species that dates back a hundred years, and which is based on the selection of promising genotypes based on the phenotype where it is expected that the offspring obtained by crossing them will exhibit desirable traits, has been very successful (Humphreys and Zwierzykowski, 2020). The first thing to do at the beginning of the meadow fescue breeding process is to determine the genetic variability of the initial breeding material, which ensures the success of the breeding process. Babić et al. (2018) found the presence of significant variability within meadow fescue wild populations and cultivars for studied morphological, productive and quality traits. The primary focus during

meadow fescue forage breeding is to create cultivars of high yield and good biomass quality. Also, drought tolerance and persistency are important criteria in these species breeding.

In this investigation, one of the aims was to determine values and genetic variability for studied traits within and among nineteen accessions of meadow fescue while in the second part of the investigation aim was to determine the dry matter yield and quality of 26 meadow fescue polycross progenies.

MATERIAL AND METHODS

Variability of meadow fescue accessions

The collection investigated included nineteen meadow fescue accessions (sixteen wild populations and three cultivars). Wild populations originated mainly from Eastern Serbia. In the study were included cultivars: Kruševački 21, Premil and Pradel. The study was performed in the experimental field of the Institute for Forage Crops Kruševac. The first part of the experiment was performed in a plant nursery at a distance plant of plant 60x60 cm. The experiment was done as a randomized block design with 30 plants per accession. The main focus was to determine values and genetic variability for heading date (HD), plant height (PH), leaf length (LL), number of vegetative (NVTP) and generative (NGTP) tillers per plant and dry matter yield per plant (DMY). HD is shown as the number of days from the first of April. All traits were measured in the first cut. DMY was measured in two cuts and presented as annual DMY. All results are shown as two-year mean values.

Data processing was done by ANOVA, based on randomized block design which was used for the determination of the mean squares of a source of variation and they were used to calculate the components of variance, according to Falconer and Mackay (1996). Broad sense heritability (h_b^2) is presented as the ratio of genetic variance to phenotypic variance. Expected genetic advance as part of the mean (GA) was calculated for each trait at 5% selection intensity ($k=2.056$) according to Falconer and Mackay (1996). Expected

genetic advance as percent of the mean (GA%) was calculated for comparison of the expected GA of traits expressed by different units. LSD test was performed to determine differences between accessions. The accessions by trait biplot (PCA) were used to help to notice relationships between analyzed traits and also accessions distribution by traits. The statistical analyses were done by the STATISTICA 8 program.

Productivity and biomass quality of meadow fescue progenies

The best plants from a nursery (60), are propagated vegetatively (from each plant cloned 30 plants) and planted in a polycross field, to a distance of 60x30 cm. After the seed was harvested from polycross, selected 26 of the best progenies were, and they were used for the second part of the trial which investigated their productivity and biomass quality. They were tested in plots 5x2 m, as a randomized block design, in 3 repetitions. The individual plot was sown with a seed quantity of 25 kg ha⁻¹. During the planting year, all plots were cut without weighing. In the next two years, two cuts were taken. The first cut was done at the beginning of the heading and the second 40 days after the first. Crude protein content (CP) was done by the Weende system (AOAC 984.13, 1990) and the content of acid detergent fiber (ADF) according to AOAC 973.18 (1997), while the neutral detergent fiber (NDF) was determined by Van Soest et al. (1991). The results are shown as two-year average values. Cluster analysis was done by the Ward method, using the Euclid distance to group meadow fescue progenies.

RESULTS AND DISCUSSION

Significant within-population variability expressed through coefficients of variation (CV%), were noted for NVTP, NGTP and DMY. Also, significant differences between meadow fescue accessions were determined for all analyzed traits (LSD) (Table 1). HD showed on average the lowest within-population variability among all traits. The difference between the earliest and the latest accessions

SNEŽANA BABIĆ ET AL.: VARIABILITY OF MEADOW FESCUE ACCESSIONS
AND PRODUCTIVITY AND QUALITY OF THEIR POLY-CROSS PROGENIES

is only 4.2 days on average. According to these results, Lemežiene and Kanapeckas (2008) did not find polymorphism in meadow fescue collection, where differences between accessions in heading were no more than 3 days on average.

PH was on average 101.1 cm, which is higher in comparison to the results of Babić et al. (2014), who found an average plant height of 89.2 cm, but significantly lower than the average plant height of 126-139 cm noted by Fang et al. (2004). LL has a positive

effect on improving dry matter quality. The LL was similar to Babić et al. (2014). NGTP in the first cut has the greatest impact on DMY, but it is also very important in terms of influencing other traits such as traits that determine seed yield. The average value for this trait of 232.7 is similar to the values given by Fang et al. (2004) where the average NGTP was 232.2, but it is significantly higher than the results obtained by Babić et al. (2014), where the average number of NGTP was 79.5.

Table 1. Average trait values and variability of meadow fescue accessions

Trait accession	HD	PH (cm)	LL (cm)	NVTP	NGTP	DMY (g/plant)
FP 1	46.5	113.8	31.0	44.7	218.4	343.4
FP 2	47.5	93.4	25.0	71.1	158.9	174.6
FP 3	43.3	109.1	25.4	81.6	289.1	233.4
FP 4	44.5	107.1	25.4	59.6	292.8	297.2
FP 5	45.4	86.0	26.5	58.3	156.7	157.7
FP 6	45.0	101.2	26.2	84.0	262.4	243.8
FP 7	44.7	103.6	25.6	88.3	303.6	228.9
FP 8	44.5	103.6	26.5	88.5	244.4	213.6
FP 9	45.4	103.5	25.7	79.4	222.6	205.5
FP 10	44.3	103.9	23.2	81.1	221.7	183.2
FP 11	44.1	108.6	23.2	63.3	260.2	213.2
FP 12	43.9	109.1	24.4	71.5	264.9	242.1
FP 13	45.0	104.1	25.4	55.9	259.7	227.3
FP 14	44.6	107.4	24.5	51.9	258.8	254.3
FP 15	44.8	92.6	25.6	78.2	308.2	234.9
FP 16	44.1	95.8	24.4	81.5	220.0	208.6
K-21	43.5	107.7	23.6	64.8	195.2	220.3
Pradel	44.0	87.2	26.1	52.9	105.8	154.6
Premil	44.2	83.9	23.8	59.1	177.6	178.9
Average	44.7	101.1	25.3	69.2	232.7	221.9
Min	42.6	80.4	17.7	18.3	99.5	112.7
Max	47.6	120.3	33.3	163.6	395.1	340.0
LSD 0.05	0.76	6.06	1.76	22.77	45.51	39.8
LSD 0.01	1.09	8.72	2.53	32.05	65.48	57.26
CV%	2.93	9.47	14.47	53.26	32.36	26.04

HD - heading date (the number of days from the first of April); PH - plant height; LL - leaf length; NVTP - number of vegetative tillers per plant; NGTP - number of generative tillers per plant; DMY - dry matter yield per plant.

The ultimate goal in the breeding program of meadow fescue, like anyone forage grasses, is to increase DMY or to improve the traits that are in a positive correlation with DMY. For the accession FP 1 was scored the highest DMY. The lowest values for this trait were determined for cultivar Pradel and accessions FP 5 and FP 2. The average DMY of the collection was 221.9 g per plant which

is significantly higher than reported by Babić et al. (2014), where the two years average DMY per plant was 90.54 g. Studying a wide collection of meadow fescue populations and cultivars, Casler and Santen (2000) determined, based on 2 years of study, that DMY was 105 g per plant on average.

The principal component biplot (Figure 1) showed correlations between the various

measured traits, which gives an opportunity for effectively determining the relationship of different traits and how the accessions were scattered across biplot quarters. DMY, like the most important trait, tended to be positively correlated with NGTP, PH and LL. The biplot shows that there is the potential to identify

accessions with high productions (FP 1 and FP 4) and also with contrasting agronomic attributes (FP 3, FP 14, FP 7, FP 12, FP 8, FP 15, FP 6, FP 13 and FP 11). Accessions FP 2, FP 5, Premil and Pradel were clearly separated from others by the lowest PH, DMY and NGTP.

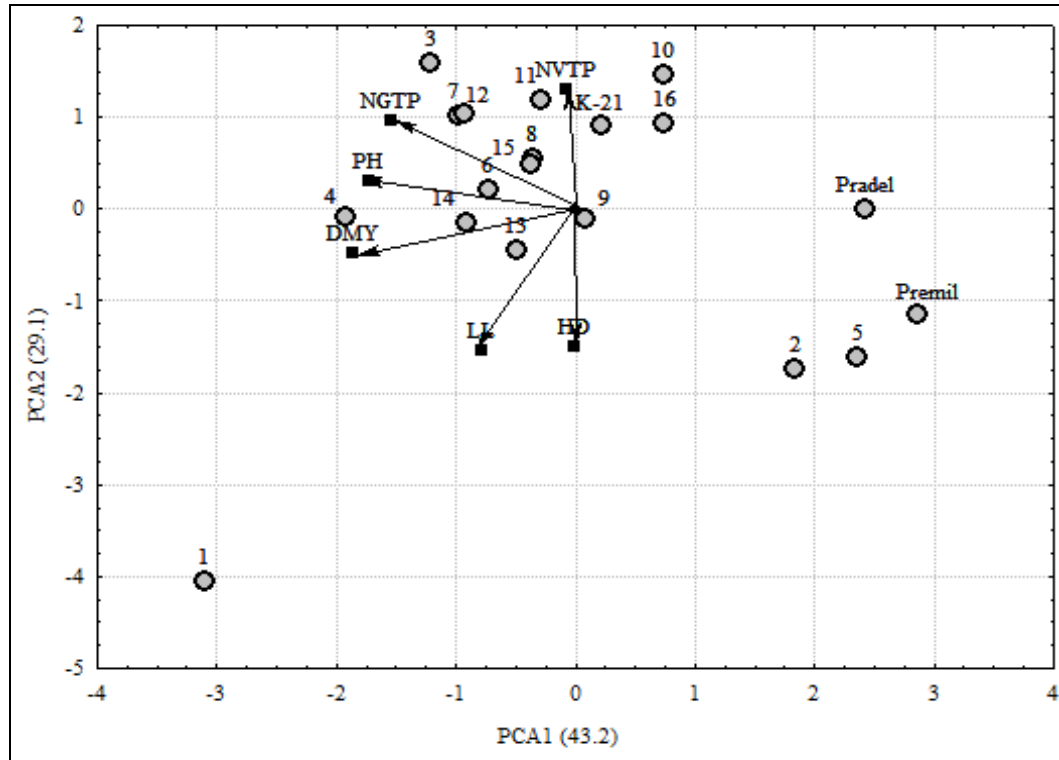


Figure 1. Accessions by trait biplot of 19 meadow fescue accessions by studied traits

High values for h_b^2 were determined for PH, HD, NGTP and DMY, while the lowest h_b^2 was in NVTP. The most important role of heritability in the research of quantitative traits is to indicate the reliability and validity of the phenotypic value as a guide to a successful breeding process (Falconer and Mackay, 1996). In general, high values for h_b^2 indicate how successful selection can be if we are based on phenotype, but its practical importance in the breeding process is further

improved if h_b^2 is accompanied by high GA.

In this case, high values for h_b^2 and GA occurred only for the NGTP and DMY, which is positive considering the fact that these traits are the most important traits in the breeding process of this species. High h_b^2 coupled with high GA for NGTP and DMY indicated that these traits are affected by the additive genes and effective selection could be made.

Table 2. Genetic parameters of studied traits

Trait	HD	PH	LL	NVTP	NGTP	DMY
h_b^2 %	74.9	78.3	59.8	26.8	70.7	68.3
σ_g^2	0.9	69.9	2.4	95.9	2639.5	1808.2
σ_f^2	1.2	89.4	4.1	358.4	3734.8	2645.8
CV g %	2.1	8.3	6.2	14.1	22.1	19.2
CV ph %	2.5	9.3	7.9	27.3	26.3	23.2
GA	1.7	14.9	2.5	10.4	89.1	71.9
GA (%)	3.8	14.8	9.8	15.3	38.3	32.4

SNEŽANA BABIĆ ET AL.: VARIABILITY OF MEADOW FESCUE ACCESSIONS
AND PRODUCTIVITY AND QUALITY OF THEIR POLYCROSS PROGENIES

The existence of variability of studied traits was determined and expressed by phenotypic (CV_{ph}) and genetic (CV_g) coefficient of variation. CV_{ph} and CV_g , as relative indicators of variability, are used to compare the variability of different traits expressed in different units of measure. For all studied traits, CV_{ph} was slightly higher than CV_g , which signified the influence of the environment to some degree in the phenotypic expression of traits. The highest CV_{ph} and analogously CV_g , as expected, were recorded for NVTP, NGTP and DMY, which indicates a higher level of genetic variability among meadow fescue accessions for these traits. The lowest values for these parameters were scored for HD which means that the meadow fescue accessions show the least variation among themselves with respect to this trait. Similar results were reported by Kanapeckas et al. (2005) and Fang et al. (2004).

In the second part of the trial, 26 meadow fescue progenies were tested on productivity expressed through DMY per ha (Table 3) and biomass quality (Table 4). High values for DMY in the first cut and total DMY were scored for progenies originating from FP 3, FP 6, FP 8 and FP 13 accessions. The highest DMY in total was found in FP 3/14 (progeny originated from 14. plant from FP 3 accession from the first part of the trial) while the lowest values for this parameter were found in FP 9/9. DMY in total was in the range from 11.71 to 14.2 tha^{-1} , DMY in the first cut ranged from 8.12 to 9.45 tha^{-1} while the values for DMY in the second cut were from 3.19 to 4.75 tha^{-1} . In opposite to this research, where the share of the first cut in the total DMY was significantly higher than the second cut, the lower DMY in the first cut and higher values for DMY in the aftermath, were reported by Kanapeckas et al. (2005).

Table 3. Dry matter yield (tha^{-1}) of meadow fescue progenies

Trait progeny	DMY I cut	DMY II cut	DMY total
FP 1/12	8.72 ^{gh}	3.64 ^{h-j}	12.36 ^{ef}
FP 1/19	8.99 ^{d-f}	3.75 ^{gh}	12.74 ^e
FP 3/6	9.34 ^{a-d}	4.74 ^a	14.08 ^{a-b}
FP 3/14	9.45 ^a	4.75 ^a	14.2 ^a
FP 4/2	8.62 ^{gh}	3.40 ^{i-k}	12.02 ^{fg}
FP 4/18	9.09 ^{b-e}	3.70 ^{g-i}	12.79 ^e
FP 6/7	9.36 ^{ab}	3.95 ^{e-g}	13.31 ^d
FP 6/11	9.30 ^{a-d}	4.24 ^{b-e}	13.54 ^{cd}
FP 6/24	9.31 ^{a-d}	4.13 ^{d-f}	13.44 ^{cd}
FP 7/3	8.43 ^{hi}	3.35 ^{i-k}	11.78 ^g
FP 8/8	9.39 ^{ab}	4.27 ^{b-d}	13.66 ^{b-d}
FP 8/18	9.41 ^{ab}	4.37 ^{b-d}	13.78 ^{a-c}
FP 8/23	9.26 ^{a-d}	4.19 ^{b-e}	13.45 ^{cd}
FP 9/9	8.37 ^{hi}	3.34 ^{i-k}	11.71 ^g
FP 10/27	8.44 ^{hi}	4.25 ^{b-d}	12.69 ^e
FP 11/19	8.70 ^{gh}	3.73 ^{gh}	12.43 ^{ef}
FP 12/2	8.58 ^{gh}	3.19 ^k	11.77 ^g
FP 13/6	9.34 ^{a-d}	4.34 ^{b-d}	13.68 ^{b-d}
FP 13/11	9.29 ^{a-d}	4.49 ^{ab}	13.78 ^{a-c}
FP 13/20	9.27 ^{a-d}	4.43 ^{bc}	13.7 ^{b-d}
FP 14/12	8.21 ⁱ	3.77 ^{gh}	11.98 ^{fg}
FP 15/4	9.24 ^{a-d}	4.44 ^{bc}	13.68 ^{b-d}
FP 15/17	9.24 ^{a-d}	4.14 ^{c-f}	13.38 ^{cd}
FP 15/22	9.03 ^{d-f}	4.25 ^{b-d}	13.28 ^d
FP 16/14	8.12 ⁱ	3.68 ^{g-i}	11.8 ^g
K-21/4	8.83 ^{e-g}	3.87 ^{f-h}	12.7 ^e
Average	8.97	4.02	12.99

Dry matter chemical composition indicates the biomass quality and it is a very important breeding criterion that is usually part of all forage breeding processes of grasses. CP content is the most important indicator of biomass quality and it largely depends on the phase in which cutting is performed and genotype (Østrem et al., 2013). Polycross progenies with the highest CP were from accessions FP 3, FP 8, FP 11, FP 12 and FP 13. Average CP in the first cut was significantly lower than the value for this trait noted by Niemelainen et al. (2000), who

scored the CP from 151 to 161 gkg⁻¹. Values for CP in the second cut were similar to Niemelainen et al. (2000).

A low level of ADF and NDF is desirable and progenies with the lowest values of them can be used in further breeding programs. ADF and NDF in forage grasses largely depend on the growth phase at the cutting, as well as the environment in which forage grasses are grown. Earlier cutting influenced lower content of ADF and NDF and therefore higher relative feed value of biomass (Ball et al., 1996).

Table 4. Chemical composition (gkg⁻¹) of meadow fescue progenies

Trait progeny	CP I cut	CP II cut	ADF I cut	ADF II cut	NDF I cut	NDF II cut
FP 1/12	121 ^{g-i}	148 ^{j-m}	343 ^{g-j}	334 ^{b-d}	596 ^a	611 ^{de}
FP 1/19	121 ^{g-i}	146 ^{l-n}	347 ^{e-g}	326 ^{fg}	589 ^{a-d}	606 ^{ef}
FP 3/6	130 ^a	155 ^{b-f}	342 ^{g-j}	318 ^h	597 ^a	616 ^{c-e}
FP 3/14	129 ^{ab}	152 ^{e-i}	355 ^{c-e}	326 ^{fg}	592 ^{ab}	626 ^{bc}
FP 4/2	119 ^{hi}	147 ^{k-n}	344 ^{g-i}	334 ^{b-d}	591 ^{a-c}	598 ^{fg}
FP 4/18	122 ^{fg}	145 ^{mn}	355 ^{c-e}	336 ^b	587 ^{b-d}	613 ^{de}
FP 6/7	128 ^{ab}	153 ^{d-h}	346 ^{fg}	324 ^{fg}	568 ^{h-j}	615 ^{c-e}
FP 6/11	122 ^{fg}	150 ^{h-k}	355 ^{c-e}	327 ^{fg}	577 ^{e-g}	613 ^{de}
FP 6/24	127 ^{b-e}	149 ^{i-l}	343 ^{g-j}	333 ^{b-d}	583 ^{d-f}	626 ^{bc}
FP 7/3	125 ^{de}	151 ^{g-k}	381 ^a	333 ^{b-d}	573 ^{gh}	608 ^{ef}
FP 8/8	129 ^{ab}	157 ^{a-c}	341 ^{g-j}	333 ^{b-d}	561 ^j	597 ^{f-h}
FP 8/18	122 ^{gh}	158 ^{ab}	342 ^{g-j}	333 ^{b-d}	576 ^{e-h}	587 ^{g-i}
FP 8/23	125 ^{ef}	155 ^{b-e}	344 ^{g-i}	332 ^{cd}	570 ^{g-i}	589 ^{gh}
FP 9/9	126 ^{c-e}	146 ^{l-n}	349 ^{d-g}	335 ^{bc}	584 ^{c-e}	656 ^a
FP 10/27	119 ⁱ	154 ^{b-f}	365 ^b	346 ^a	575 ^{f-h}	620 ^{b-d}
FP 11/19	128 ^{a-c}	154 ^{b-f}	376 ^a	334 ^{b-d}	569 ^{g-i}	613 ^{de}
FP 12/2	129 ^{ab}	148 ^{j-m}	360 ^{bc}	348 ^a	570 ^{g-i}	628 ^b
FP 13/6	128 ^{a-c}	158 ^{ab}	337 ^{ij}	331 ^{de}	564 ^{ij}	567 ^{jk}
FP 13/11	127 ^{b-e}	159 ^a	335 ^j	327 ^{ef}	569 ^{g-i}	592 ^{gh}
FP 13/20	129 ^{ab}	157 ^{a-c}	342 ^{g-j}	334 ^{b-d}	573 ^{gh}	587 ^{hi}
FP 14/12	125 ^e	154 ^{b-f}	366 ^b	334 ^{b-d}	568 ^{h-j}	598 ^{fg}
FP 15/4	130 ^a	156 ^{a-d}	346 ^{f-h}	316 ^{hi}	567 ^{h-j}	565 ^k
FP 15/17	128 ^{a-c}	149 ^{i-l}	345 ^{g-j}	323 ^g	574 ^{gh}	565 ^k
FP 15/22	127 ^{b-e}	151 ^{f-j}	338 ^{h-j}	324 ^{fg}	571 ^{g-i}	564 ^k
FP 16/14	120 ^{g-i}	147 ^{k-n}	346 ^{f-h}	314 ⁱ	576 ^{e-h}	569 ^{jk}
K-21/4	125 ^{ef}	144 ⁿ	347 ^{e-g}	324 ^{fg}	570 ^{g-i}	576 ^{ij}
Average	125.4	157.7	349.6	330.0	576.5	600.2

CP - crud protein, ADF - acid detergent fiber, NDF - neutral detergent fiber.

Cluster analysis was performed in order to group and separate superior progenies. Progenies were clustered into six groups (Figure 2). The first group was composed of progenies with low DMY and low CP. The second, third and fourth groups consisted of the best progenies which are characterized by

the highest DMY and CP while the ADF and NDF are lower than in the rest and they could be used as a promising material in future breeding programs. Also, progenies grouped in the fifth group (low DMY but high CP), could be interested in some breeding programs focused on quality improvement.

SNEŽANA BABIĆ ET AL.: VARIABILITY OF MEADOW FESCUE ACCESSIONS
AND PRODUCTIVITY AND QUALITY OF THEIR POLY-CROSS PROGENIES

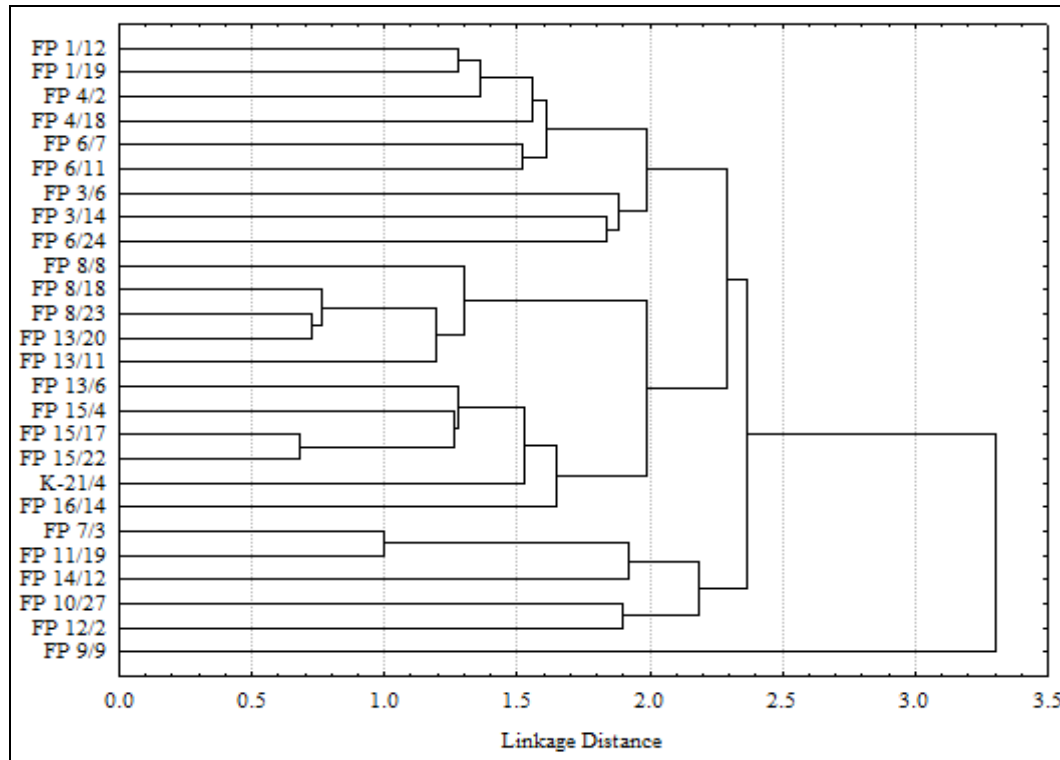


Figure 2. Grouping of meadow fescue progenies based on productivity and quality traits

CONCLUSIONS

This study indicated that in investigated material was enough variation for the most important traits which was confirmed by analysis of variance. The highest within accession variability expressed through coefficients of variation was found for a number of vegetative and generative tillers per plant and dry matter yield, while the lowest variability showed heading date. PCA biplot showed that dry matter yield positively correlated with the number of generative tillers per plant, plant height and leaf length. High values of broad sense heritability for a number of generative tillers per plant and dry matter yield per plant coupled with high genetic advance as percent of the mean, indicating that these traits were affected by additive genes and effective selection could be made for these characters in these collections. In the second part of the study, based on the obtained results for yield and quality characteristics, the progenies with the highest yield and the best quality are distinguished from the others. The cluster diagram clearly separated progenies with the highest dry

matter yield and crude protein content and lowest ADF and NDF content which could be used as a promising material in future breeding programs of meadow fescue.

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