

## STRUCTURAL ANALYSIS AND SEED PRODUCTIVITY OF POPULATIONS OF BIRD'S FOOT TREFOIL GROWN UNDER CONDITIONS OF CENTRAL NORTHERN BULGARIA

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### Abstract

During the period 20016-2018 in the experimental field of RIMSA-Troyan were tested selection populations of bird's foot trefoil *Syn*<sub>1</sub>, *Syn*<sub>2</sub>, *Syn*<sub>3</sub>, *Syn*<sub>4</sub>, *Syn*<sub>5</sub>, compared with the Bulgarian variety 'Targovishte 1'. Seed yield and its structural elements were determined in the second and third years of the development of grassland and on the basis of studied reproductive indicators interrelations among them and productivity were established. It was found that the maximum yield of seeds was obtained by the population *Syn*<sub>1</sub> - 0.37 t .ha<sup>-1</sup>, with an excess over the standard by 23.73%. High productivity is determined by the large number of seeds per pod (18.6). The lowest degree of variability according to the variation coefficients was found in the number of racemes per stem (5.89), and the highest in the number of pods per plant (19.40). The established very strong correlation between the number of pods per raceme and the weight of 1000 seeds ( $r = 0.7939$ ), allows to derive the regression equation -  $y = 0.0794x + 0.9588$ . Thus, the presented statistical analysis of the data from the seed productivity allows to make a complete characterization of the populations, which will serve for the further selection process.

**Key words:** *Lotus corniculatus* L., seed yield, structural elements, correlation coefficients Regression dependence.

### INTRODUCTION

Bird's foot trefoil (*Lotus corniculatus* L.) is an excellent component for creating artificial meadows and pastures (Bozhanska, 2017). It is considered as one of the most important fodder legumes along with alfalfa and clover (Bozhanska & Naydenova, 2020; Vasileva, 2017; Naydenova & Bozhanska, 2020). Variation in the quantitative and qualitative characteristics of the genetic resources of plants grown in different habitats is important for seed productivity (Kyuchukova, 2009; Uzun et al., 2015).

In the last few years, bird's foot trefoil has gained popularity due to its nitrogen-fixing ability (Vasileva & Ilieva, 2017), which is important for reducing nitrogen costs and defines its cultivation as economically efficient (Waghorn, 2008). On the other hand, its resistance to pathogens reduces rooting of roots (Beuselinck et al., 2005). A disadvantage of bird's foot trefoil is its low seed productivity (Garcia-Diaz & Steiner, 2000). This is due to the uneven cracking of the pods, which

prevents its spreading (Sareen, 2004). The seed yield of bird's foot trefoil depends on the duration of sunlight and therefore the long summer days in some parts of the world are ideal for its production (Ferguson, 2017). The determined negative correlation among some characteristics (yields of dry matter and seeds, morphological, as well as disease resistance) and the different altitudes of collected seeds prove significant inherent variations in all traits and thus determine the huge genetic diversity of the region (Uzun & Donmez, 2016).

The objective of the present research was to study 5 bird's foot trefoil populations and to determine the seeding potential of their seeds, their structural elements determining yield and the relationship among them.

### MATERIALS AND METHODS

During the period 2016-2018 in the experimental field of RIMSA-Troyan were tested selection populations of bird's foot trefoil, compared with 'Targovishte 1' - a Bulgarian cultivar: *Syn*<sub>1</sub> (limited free

pollination of genotypes of 'Bright' and 'Georgia 1' cultivars), Syn<sub>2</sub> (pre-pollinated plants of local population with an origin from the village of Staro Selo and the Hungarian cultivar 'Ergechi'), Syn<sub>3</sub> (synthetic population of local genotypes originating from Central Northern Bulgaria), Syn<sub>4</sub> (synthetic population of the Hungarian cultivars 'Pecoli' and 'Gjiki'), Syn<sub>5</sub> (synthetic population of genotypes originating from Shumen, Nessebar, Kiten) Sowing was carried out in 2016, and the seeds were harvested over the next two years (2017 and 2018).

The sowing was performed manually, at a depth of 0.5-1.0 cm and a sowing rate of 12.0 t ha<sup>-1</sup>. The phosphorous and potassium fertilizers were applied as reserve at a rate of 240 t ha<sup>-1</sup> a. i., while nitrogen was applied at one fertilization with 60 t ha<sup>-1</sup> a. i., before sowing. The birds foot trefoil was cultivated under the agricultural techniques, adopted in the area. The seeds were harvested from second cuttings as 65-70% of them became ripen.

The following characteristics were observed from the structural elements of the seed yield: number of racemes per plant; number seeds per pod; number of pods per plant; seed yield (t ha<sup>-1</sup>). The study included average data of the different characteristics over the years. The mean values (X), minimum (min) and maximum (max) limits of the structural elements and seed

yield were calculated (Lidanski, 1988). The degree of variation (VC) of parameters was determined through variation coefficient according to the scheme of Mamaev: up to 7% - very low, 7.1 to 12.0 % low, 12.1 to 20.0 % moderate; 20.1 – 40.0 % high: over 40.0 % - very high. Correlations (r) of Brave and Pirson were calculated to prove the relations between the different characteristics and their impact on productivity as well as among them. The data was processed by Microsoft Excel. The significant differences were determined by the methods of dispersion analysis.

## RESULTS AND DISCUSSIONS

The 2016 is considered to be favorable in terms of meteorological conditions (Table 1), when the precipitation was evenly distributed during the months of the vegetation period. The average daily air temperature of 11.1 C° and the amount of precipitation of 115.2 l/m<sup>2</sup> in April in the year of sowing (2016) had a favourable effect on the normal sprouting of bird's foot trefoil. Phenophase 2-4 leaves occurred normally in mid-April, and bud formation in mid-June, when the first regrowth was harvested. The low soil moisture due to rainfall amount of 56.9 and 42.2 l/m<sup>2</sup> in June and July slowed down the growth and development of bird's-foot-trefoil and the formation of a second regrowth.

Table 1. Climatic characteristic for the period of 2016-2018

Years	Monthly precipitation amount (l/m <sup>2</sup> )												Average temperature	Average precipitation amount
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII		
2016	88,8	59,9	58,5	115,2	161,5	56,9	42,2	82,8	26,2	51,3	46,9	46,8	837,0	594,6
2017	70,1	21,1	60,7	90,4	133,1	113,2	186,6	13,2	38,9	126,3	53,7	75,9	983,2	762,4
2018	29,6	96,9	83,2	22,8	82,5	174,3	241,1	9,4	30,0	56,2	45,2	48,2	919,4	699,5
Years	Average monthly air temperature (°C)												Average precipitation amount	Average precipitation amount
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII		
2016	-1,0	7,4	7,4	13,4	14,0	20,0	21,1	20,0	16,2	9,5	5,4	-0,7	11,1	15,2
2017	-5,3	2,1	8,1	10,1	14,5	19,9	20,6	20,4	16,4	9,8	6,3	3,5	10,5	15,0
2018	1,5	1,7	5,1	14,5	16,7	18,7	20,4	20,3	15,8	11,2	4,9	1,0	11,0	15,3

The second year of plant development (first seed productive year) was characterized by a significantly higher amount of precipitation in June (113.2 l/m<sup>2</sup>), July (186.6 l/m<sup>2</sup>), when the seed regrowth is formed. The lower humidity in August accelerated the harvesting of seeds, which was done during the first ten days of the

month, but did not prevent high seed productivity from the grassland.

The agrometeorological characteristics of the third year of grassland development and the second for seed harvesting are quite different from the other two.

The significantly higher amount of precipitation in June (174.3 l/m<sup>2</sup>), July (241.1 l/m<sup>2</sup>) is obvious, as a very low moisture content was registered in August (9.4 l/m<sup>2</sup>) and high average monthly air temperature (20.3°C). This

was a prerequisite for the rapid cracking of the seeds and obtaining a lower yield.

The average annual results and the average ones on the structural analysis of seed yield for the whole study period are shown in Table 2.

Table 2. Structural elements of the seed yield year and per Mean for the period

populations	Number of pods per racemes			Number of racemes per stems			Number of seeds in pod			1000 seed weight		
	2017	2018	Mean	2017	2018	Mean	2017	2018	Mean	2017	2018	Mean
<i>Targovishte 1</i>	3,1	2,9	3,0	12,9	10,8	11,9	15,3	19,4	17,4	1,08	1,24	1,16
Syn <sub>1</sub>	3,8	3,5	3,7	13,5	11,2	12,4	16,4	20,8	18,6	1,25	1,31	1,28
Syn <sub>2</sub>	3,5	3,3	3,4	13,3	11,8	12,6	12,7	17,3	15,0	1,14	1,28	1,21
Syn <sub>3</sub>	3,0	3,3	3,2	13,3	13,5	13,4	19,5	15,1	17,3	1,25	1,18	1,22
Syn <sub>4</sub>	4,1	4,6	4,4	14,6	12,1	13,4	20,4	14,7	17,6	1,50	1,25	1,38
Syn <sub>5</sub>	4,8	4,9	4,9	14,9	12,8	13,9	17,1	13,3	15,2	1,30	1,28	1,29
X	3,72	3,75	3,73	13,75	12,03	12,89	16,90	16,77	16,83	1,25	1,26	1,26
SD	0,67	0,80	0,72	0,80	1,00	0,76	2,81	2,92	1,42	0,15	0,05	0,08
VC	18,12	21,45	19,40	5,85	8,32	5,89	16,63	17,40	8,46	11,61	3,58	6,06
Min	3,00	2,90	3,00	12,90	10,80	11,85	12,70	13,30	15,00	1,08	1,18	1,16
Max	4,80	4,90	4,85	14,90	13,50	13,85	20,40	20,80	18,60	1,50	1,31	1,38

The impact of all factors involved in the experiment reflects on the structural elements of seed yield. In the first seed production year, data show that Syn<sub>5</sub> population had the highest number of pods per racemes - 4.8 and number of racemes per stem - 14.9, compared to other bird's foot trefoil populations tested. The difference in the values of the number of pods per racemes is insignificant in terms of the standard and populations Syn<sub>1</sub>, Syn<sub>2</sub>, Syn<sub>3</sub>. The value of this indicator is 1 point higher in Syn<sub>4</sub> population. Of the indicators determining the structural seed yield, the number of pods per racemes had the highest degree of variability, which can be seen from the coefficient of variation (CV = 18.12). The values for the number of racemes per stem vary within the narrowest limits in the individual populations, respectively from 12.9 to 14.9, which is due to their low coefficient of variation CV = 5.85. The mean value for the number of pods per racemes of the test populations was X = 3.72 with a standard deviation SD = 0.67. The average number of racemes per stem was 13.75, and the standard deviation SD = 0.80. The highest number of seeds per pod was found in Sin<sub>1</sub> population, as degree of variability according to the variation coefficient (CV=16.63) was average. A minimum value was reported in Syn<sub>2</sub> population. The mean value for this indicator is X = 16.90 and the standard deviation is 2.81. The weight of 1000 seeds per year in all tested populations varied

from 1.08 to 1.50. The degree of variability is low, which is due to the insignificant difference between the minimum (1.08) and maximum values (1.50). The mean value of the number of seeds per pod is X = 1.25, and the standard deviation SD = 0.15.

In the second seed production year, the number of beans per racemes was from 2.9 in the standard to 4.9 in Syn<sub>5</sub> population, with an mean value of X = 3.75. This indicator has the highest value of the coefficient of variation (VC = 21.45) compared to all indicators characterizing seed productivity. In general, there is some correspondence between Number of pods per racemes and Number of racemes per stems over the years of study. All populations, slightly exceeded the standard in the number of racemes per stem. On this basis, however, the variation is within narrow limits (VC = 8.32). The average data for the year show that the populations formed an average of 16.77 Number of seeds per pod with a minimum value of 13.30 and a maximum of 20.80. The average weight of 1000 seeds were 1.26 g, which is a very low variability according to the coefficient of variation VC = 3.58 and the standard deviation SD = 0.05. The low values of seed weight are due to the fact that the seed undergrowth was formed in the absence of precipitation in August (9.4 l/m<sup>2</sup>), which impeded the increasement of their weight.

The highest average number of pods per plant and number of racemes per stem was registered in population Syn<sub>5</sub>, respectively 4.9 and 13.9. The number of pods per plant had an average degree of variability according to variation coefficient CV% - 19.40, and the number of racemes per stem had a very low degree of variability - 5.89. The highest number of seeds per pod was in Syn<sub>1</sub> population (18.6), while the highest weight of 1000 seeds was registered in Syn<sub>4</sub> population (1.38 g). There were not any significant differences in the number of pods per plant and the number of racemes per stem in the tested populations compared to the standard. The lowest degree of variability according to the variation coefficients was found in the number of racemes per stem

(5.89), and the highest one in the number of pods per plant (19.40).

The values for seed yield in the studied cultivars and populations (Table 3) varied widely over years and on average for the study period. On average for the study period the seed yield in the tested populations, with the exception of the population Syn<sub>2</sub>, exceeded the standard cultivar 'Targovishte 1'. The highest seed yield was obtained from population Syn<sub>1</sub> - 0.37 t.ha<sup>-1</sup>, as the excess compared to the standard was 23.73%. A relatively high yield was also shown by Sin<sub>3</sub> population, which exceeded the standard by 11.54%. Syn<sub>1</sub> population had very well-proven differences in seed yield. Good evidence of differences was also reported in the yield of Syn<sub>3</sub> population.

Table 3. Seed yield year and per Mean for the period, t ha<sup>-1</sup>

populations	2017 r.		2018 r.		Mean for the period	
	t ha <sup>-1</sup>	% to St	t ha <sup>-1</sup>	% to St	t ha <sup>-1</sup>	% to St
<i>Targovishte 1</i>	0.29	100.00	0.31	100.00	0.30	100.00
Syn <sub>1</sub>	0.38	129.91+++	0.36	117.90++	0.37	123.73+++
Syn <sub>2</sub>	0.30	102.56-	0.28	91.13-	0.29	96.68-
Syn <sub>3</sub>	0.34	118.29+	0.32	105.16-	0.33	111.54+
Syn <sub>4</sub>	0.32	111.79-	0.32	103.23-	0.32	107.39-
Syn <sub>5</sub>	0.33	114.70+	0.28	91.77-	0.31	102.90-
LSD 5%	0.37	13.58	0.33	10.69	0.24	8.25
LSD 1%	0.55	18.80	0.45	14.80	0.34	11.43
LSD 0.1%	0.75	25.94	0.63	20.42	0.47	15.76

LSD – Fisher's least significant difference

Seed yield in the first seed production year varied from 0.30 t ha<sup>-1</sup> to 0.38 t ha<sup>-1</sup>. The maximum value was obtained in the population Syn<sub>1</sub>, as the excess compared to the standard was 29.91%. All tested populations exceeded the seed yield standard. The insemination of plants in the second year of their development could be explained by the formation of a larger number of generative stems. The excess is from 2.56 to 29.91%. The low seed yields that year were due to unfavorable weather conditions. The seed yields in Syn<sub>4</sub> and Sin<sub>5</sub> populations are approximately the same, 0.32 and 0.33 t ha<sup>-1</sup>, respectively.

In the second year, the seed productivity in most of the populations was lower than the first year. The highest seed yield was gathered from population Syn<sub>1</sub> (0.36 t ha<sup>-1</sup>), which exceeded the standard by 17.90%. The high seed yield in the second year was due to the good supply of moisture of the grassland in June and July and

the higher values for the number of seeds per pod and the number of racemes per stem. Seed yield exceeding the standard was reported in Syn<sub>3</sub> and Syn<sub>4</sub> populations (0.32 t ha<sup>-1</sup>). In Syn<sub>1</sub> population, the differences are well established. In all other populations, there was a negative proof of the differences.

Populations from different habitats play a key role in terms of their main characteristics - adaptability and resistance to biotic and abiotic stress. Due to the impact of extreme weather conditions on the adaptability and productivity of bird's foot trefoil, according to Churkova (2013) the synthetic population that serves as a starting material for selection should be determined before it is approved as a cultivar in order to show its potential before spreading. The different yields obtained over the years show that the populations have shown their productive potential depending on the climatic

conditions during the years of study as a result of their origin.

Theoretically, each plant has the potential to produce seeds equivalent to fertile ova. Of the five populations studied, it was found that none of the populations produced as many seeds as expected. The difference between the theoretical and the actual seed yield is large (Churkova, 2002). Pods cracking is a serious problem in bird's-foot-trefoil. The development of the seeds and the cracking of the pods depend on the relative air humidity, as the most important factor. The period of pod cracking is related to the maturity of the legumes, environmental conditions and individual genetic differences of plants (Sareen, 2004). The difference in the yield of the studied populations can be attributed to the uneven flowering period, due to which during the harvesting of seeds all pods do not have the same stage of maturity. In the present study, all populations began to bloom in late June in both years, but due to different weather conditions over the years, the seeds in the first production year was harvested in early August and for the second year in the last ten days of the same month. Other factors that could be a

prerequisite for low seed yields are abortion of flower buds, lack of pollination and genetic factors. The presented structural analysis shows the great variability of the indicators in the individual populations. In the future, this can be used to isolate lines with less pod cracking and higher seed yield.

The performed correlation data analysis (Table 4) shows that the seed yield is positively correlated with all indicators of its structural elements. A strong correlation was found between seed yield and number of seeds/ pods ( $r = 0.7690$ ).

Very strong correlation was established between the number of pods per racemes and the weight of 1000 seeds ( $r = 0.7939$ ), which allows to derive the regression equation -  $y = 0.0794x + 0.9588$  (Figure 1) and a strong correlation between the number of racemes per stem and number of seeds per pod ( $r = 0.7462$ ). A good positive correlation was observed between the weight of 1000 seeds and the number of racemes per stem ( $r = 0.6052$ ). A slight positive correlation of seed yield with Number of pods / raceme ( $r = 0.0297$ ) and Number of racemes per stems ( $r = 0.0023$ ) was found.

Table 4. Correlation coefficients between the Seed yield and Structural elements

indicators	Seed yield	Number of pods per racemes	Number of racemes per stems	Number of seeds in pod	1000 seed weight
Seed yield	1				
Number of pods per racemes	0.0297	1			
Number of racemes per stems	0.0023	0.7462	1		
Number of seeds in pod	0.7690	-0.2849	-0.3558	1	
1000 seed weight	0.3449	0.7939	0.6052	0.1719	1

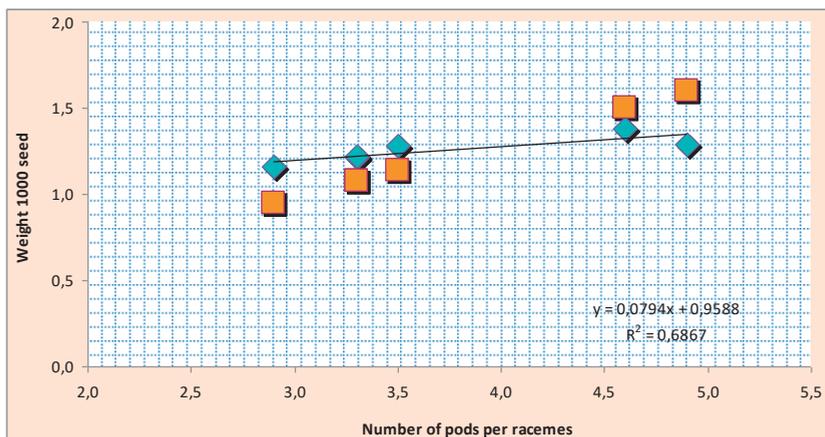


Figure 1. Regression dependence between 1000 seed Weight (g) and the Number of pods per racemes (Number)

## CONCLUSIONS

Under the conditions of light gray pseudo-podzolic soils, from the studied 5 bird's-foot-trefoil population, the maximum seed yield was achieved by population Syn<sub>1</sub> - 0.37 t.ha<sup>-1</sup>, with an excess over the standard by 23.73%. High productivity is determined by the large number of seeds per pod (18.6). The lowest degree of variability according to the variation coefficients was found in the number of racemes per stem (5.89), and the highest in the number of pods per plant (19.40). The established very strong correlation between the number of pods per raceme and the weight of 1000 seeds ( $r = 0.7939$ ), allows to derive the regression equation -  $y = 0.0794x + 0.9588$ . The obtained data show that the genetic capabilities of the populations are best expressed in a certain area, with a combination of appropriate soil and climatic conditions and are important for further selection work.

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