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RESEARCH REGARDING THE INFLUENCE OF SEED SIZE ON ROOT NODULE FORMATION CAPACITY IN COMMON BIRD'S-FOOT-TREFOIL (*Lotus corniculatus* L.)

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Abstract

In the process of biological fixation of atmospheric nitrogen, root nodule growth and development in legumes is the result of the symbiosis relationship between these plant species and nitrogen fixing bacteria. In this respect, research points out the effect of common bird's-foot-trefoil (Lotus corniculatus L.) seed size on root nodule formation capacity in the presence of nitrogen fixing bacterium Mesorhyzobium loti. Our results show that there is a direct relationship between seed size and dimensional features of root nodules in common bird's-foot-trefoil (number of nodules/plant, and weight of nodules).

***Key words**: Lotus corniculatus L., root nodule formation capacity, nitrogen fixing bacteria, seed size.*

INTRODUCTION

Bird's-foot-trefoil (*Lotus corniculatus* L.) is a grassland legume species with a high degree of rusticity due to both its ecological plasticity and specific biological features (Ross et al., 1985). The germination capacity of (*Lotus corniculatus* L. is influenced by physical factors and by seed size (Toth, 2013).

The efficiency of the relationship of symbiosis between the plants of bird's-foot-trefoil and specific bacteria of *Mesorhyzobium meliloti* is strongly influenced by natural conditions, by the diversity of existing bacterial strains in the soil and by some morpho-physiological features of the species (Jarvis et al., 1982; Monza et al., 1992; Irisarry et al., 1996; Baraibar et al., 1999; Rebah et al., 2002; Sotelo et al., 2011).

In this paper, the authors present the results of research carried out on the influence of seed size on root nodule formation in bird's-foot-trefoil.

MATERIALS AND METHODS

Research was carried out in 2014, in a vegetation house, using as biological material seeds of the Nico cultivar of *Lotus corniculatus* L. Before sowing, the seeds from the crop of 2013 were grouped into five size groups depending on seed diameter: a1 - very small seeds (Ø=0.50-1.00 mm), a2 - small seeds (Ø=1.01-1.25 mm), a3 - medium-size seeds (Ø=1.26-1.40 mm), a4 - large seeds (Ø=1.41-1.50 mm) and a5 - very large seeds (Ø> 1.51 mm). We sowed from each seed group several seeds in vegetation vases and after sprouting we preserved only ten plants per vase that were studied individually during the vegetation period.

During the vegetation period, we made in each plant quantitative measurements regarding the plant growth and development: height, ramification, sprouting, and weight.

The nodule formation capacity was determined at the beginning of the blooming period in ten plants per group. After 12 h of immersion, we removed the soil using a mild flow of water and the nodules were sampled from the root system and collected in Petri dishes on a substratum of porous paper to absorb the water and then weighed with a digital laboratory balance, BP 221 S (Sartorius, Germany), with a measuring accuracy of 0.1 mg.

In order to facilitate the measurements of the number and size of nodules, they were photographed with a digital camera and the images were processed using the Image J software (Schneider et al., 2012) (File - Open; Process - Enhance contrast / Subtract Background; Image - Adjust - Colour Threshold; Analyze - Analyze Particles).

The nodules on each plant were grouped into three size groups - small, medium and large – using Microsoft Excel (Home - Sort & Filter).

The data thus obtained (number, weight and size of nodules) were processed statistically with variance analysis and the Duncan Test. In order to point out relationships between seed size and nodule formation capacity, the authors also carried out a study of the correlations between size-related features of seeds and nodules.

Nodule observations and measurements were also evaluated through the nodule index calculus (Rebah et al., 2002) with the formula:

$$\text{Nodule index} = A \times B \times C \leq 18,$$

where:

- A- nodule size (1- small, 2- medium, 3- large);
- B- nodule colour (1- white, 2- pink);
- C- nodule number (1- few, 2- medium, 3- many).

RESULTS AND DISCUSSIONS

The biological features in plants and the natural conditions during fructification and harvesting of bird's-foot-trefoil seeds influence strongly seed size and weight. This feature can affect, in a certain measure, the vigour of new bird's-foot-trefoil plants, including the nodule formation capacity, which depends on the nitrogen nutrition rate (from the symbiosis process with nitrogen fixing bacteria) (Sotelo et al., 2011).

Data obtained and presented in Table 1 show that the number of nodules/plant reached values between 135 and 386 depending on seed size, with seed weight ranking between 22-212 mg/plant. The largest number of nodules (386/plant) and the largest weight of nodules (212 mg/plant) were in the medium-size seed group (Ø=1.51-1.60 mm) (191 nodules/plant). At the extreme limits of seed size groups (<1.25 and >1.51 mm), the size values of the nodules were smaller and insignificant statistically. Likewise, the efficiency of nodules established through the measurement of the nodule index is larger (12) in the large size group (ranging between 1.26 and 1.60 mm).

Table 1. Influence of *Lotus corniculatus* L. seed size on nodule formation capacity. Size groups are: a1 – very small seeds (Ø=0.5-1.0 mm), a2 – small seeds (Ø=1.01-1.25 mm), a3 – medium seeds (Ø=1.26-1.40 mm), a4 – large seeds (Ø=1.41-1.50 mm), a5 – very large seeds (Ø=1.51-1.60 mm)

Seed size groups	Nodule number per plant	Nodule weight (mg/ plant)	Nodule efficiency			
			A	B	C	Nodule index (AxBxC)
a1	150	22	1	2	2	4
a2	135	35	1	2	2	4
a3	386***	212***	2	2	3	12
a4	191***	135***	3	2	2	12
a5	135	43	3	2	2	12
DL 5%	17.03	27.35	-	-	-	-

According to the experimental protocol, the nodules were grouped, in their turn, into three size groups – small, medium, and large. Thus, in the very small seed group, the share of nodules per size groups was as follows: 50% small nodules, 48% medium nodules and only 2% large nodules. In the larger seed groups, the share of large nodules increases to 16-20% and the share of small and medium nodules decreases (Table 2).

Table 2. Influence of *Lotus corniculatus* L. seed size on nodule size. Size groups are: a1 – very small seeds (Ø=0.5-1.0 mm), a2 – small seeds (Ø=1.01-1.25 mm), a3 – medium seeds (Ø=1.26-1.40 mm), a4 – large seeds (Ø=1.41-1.50 mm), a5 – very large seeds (Ø=1.51-1.60 mm)

Seed size groups	Small nodules (%)	Medium nodules (%)	Large nodules (%)
a1	50	48	2
a2	59*	31°°°	10**
a3	44	36°°°	20***
a4	54	30°°°	16***
a5	74	35°°°	18***
DL 5%	7.65	5.43	4.44

As shown in Table 3, there is a relationship between seed size and weight, and the nodule formation capacity in bird's-foot-trefoil.

From this perspective, the authors noted a close correlation between both seed size and medium-size nodule share (r=0.94*) and seed size and nodule number (r=45) and nodule weight (r=0.59). As for seed weight (MMB), the higher values of correlation coefficients were in the correlations with the number of nodules (r=0.69) with nodule weights (r=0.82) and with the share of medium-size nodules (r=0.87).

Table 3. Correlation coefficients between *Lotus corniculatus* L. seed size and weight and nodule formation capacity

Specification	Nodule number	Nodule weight	Large nodules	Medium nodules	Small nodules
Seed size (Ø)	0.45	0.59	0.65	0.94*	0.43
Seed weight (MMB)	0.69	0.82	0.42	0.87	0.22

CONCLUSIONS

In general, bird's-foot-trefoil seed size correlates with the nodule formation capacity expressed as the number and size of the nodules.

In plants obtained from very small, and small seeds, the share of large nodules was of only 2-10% compared to plants stemming from the large seeds with 16-20% large nodules.

There is a stronger correlation between seed size and weight and the share of medium-size nodules (r= 0.94 and 0.87).

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