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PRE-SOWING SEED INOCULATION IN THE BIRDSFOOT TREFOIL SEED PRODUCTION

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ABSTRACT

A prerequisite for the improvement of birdsfoot trefoilproduction is the production of sufficient quantities of good quality seeds. The aim of the study was to analyze the impact of the seed inoculation with mesorhizobial bacteria on yield and yield components of birdsfoot trefoil seed (*Lotus corniculatus* L.). In experiment birdsfoot trefoil cultivars Zora, K-37 and Rocco, and two strains of *Mesorhizobium loti* were used. A trial was carried out in spring 2012 on a private estate in the village of Ivanovci near Ljig (Serbia) and were set up on brown forest soil type, using a randomized block design with three replications and plot size of 5 m (5x1m). Seed inoculation was performed immediately before sowing. Sowing was carried out at a distance of 20 cm row spacing with the amount of seed of 10 kg ha^{-} . Irrespective of inoculation, cultivars significantly differed in terms of the number of stems m^{-}. Two-factorial experiment showed significant influence of *M. loti* strains as well as interaction between the strains and the cultivars on seed yield and yield components investigated (with exemption on seed number per pod). Generally, both strains of *M. loti* have had a positive impact on yield components and seed yield of the birdsfoot trefoil cultivars. Inoculation of seeds has significantly influenced the increase ofthousand seeds weight in the cultivar Rocco, number of stems m^{-} and seed yield of cultivars Zora and Rocco.

Key words: *birdsfoot trefoil, inoculation, seed yield, Mesorhizobium loti.*

INTRODUCTION

In the area of Southeast Europe in less favourable growing conditions, especially in terms of climate and soil, to produce sufficient quantities of forage, a special attention is given to the cultivation of birdsfoot trefoil (*Lotus corniculatus* L.). In The Republic of Serbia there are no reliable statistics on yields and areas where the trefoil is grown, although it is believed that among the perennial legumes by abundance it takes the third place, after alfalfa and red clover (Đukić et al., 2007). It is particularly important species in the hilly and mountainous regions of Serbia (Petrović et al., 2011). A precondition for the improvement of production of

birdsfoot trefoil is the pro-duction of sufficient quantities of quality seeds. According to Gullien (2007) the average yields of birdsfoot trefoil at a global level are below 200 kg ha^{-}. According to Miladinović (1967) with full agricultural technology seed yields of birdsfoot trefoil in our conditions can reach more than 350 kg ha^{-}. Analyzing the seed yield of birdsfoot trefoil in three locations McGrew et al. (1986b) found that there are significant interactions genotype x environment, so as to test the potential for seed yield should be carried out in an environment where the seed is commercialized. According to Steiner et al. (1995) the seed yield of perennial legumes is mainly determined by the genetic base of the cultivar, environmental conditions of the area, time of cutting the first cut, the presence of insect pollinators, as well as the interaction of genotype/environment. In providing legumes with nitrogen, the symbiotic nitrogen fixation plays an important role, which can be realized in symbiosis of legumes with the soil bacteria from the family *Rhizobiaceae* (Marinković et al., 2010; Delić et al., 2013). On the activity of these bacteria affects the physical and chemical properties of soils particularly soil pH, climatic factors, seasons, agricultural technology, especially fertilization as well as biotic factors (host plant, plant cover, plant deseases, insects, viruses, nematodes) (Bašić, 2014; Delić, 2014). Application of inoculation of nodule bacteria *Mesorhizobium loti* before sowing stimulates root nodulation in legumes and affects better supply of plants with nitrogen (Jarvis et al., 1982; 1997). The aim of the study was to analyze the impact of sowing seed inoculation with bacteria *M. loti* on the yield components and seed yield of birdsfoot trefoil.

MATERIALS AND METHODS

The experiment was set up in the village of Ivanovci near municipality of Ljig (Serbia) 2012 on the cambisol soil type (according to the WRB classification), with the medium amount of nutrients. The main tillage was carried out to a depth of 30 cm. Together with the main tillage, the soil was entered with 300 kg ha^{-} $N_{15}P_{15}K_{15}$. Two factorial experiment was set up as a completely randomized block design with three replications and plot size of 5m (5x1m). Three cultivars of birdsfoot trefoil were used: K-37 (The Institute for Forage Crops Kruševac), Zora (Institute for Agricultural and Technological Research, Zaječar) and Rocco (Italian cultivar). In addition, two *M. loti* strains (608 and Z) used as plant inoculants. *M. loti* strain 608 isolated from the root nodules of the plant *Lotus corniculatus* from the pasture on the mountain Ozren, a collection of strains of the Institute of Soil Science, Belgrade while *M. loti* strain Z isolated from the soil at the site in Eastern Serbia, a collection of strains of the Institute of Soil Science, Belgrade. The cultivars were inoculated with single mesorhizobium strains resulting in inoculated treatmants, R_1 (strain 608) and R_2 (strain Z) which were compared with controled treatmants (uninoculated cultivars). Seed inoculation was performed immediately before sowing. Sowing was done on row spacing of 20 cm, with a quantity of seeds 10 kg ha^{-}. Weed control was done mechanically on two occasions. The crop was grown without irrigation.

Yield and seed yield components were determined from the second growth in the second year of cultivation when the seed production is usually performed. The

following yield components were determined: number of stems m^{-} (counting the area of 0. 2 m per elementary plot), number of pods per stem (counting on ten randomly selected stems from the elementary plot). In laboratory were determined: the number of seeds per pod (a sample of ten randomly selected inflorescences per elementary plot) and thousand seed weight (based on the weight of 5x100 of the seed). The potential seed yield is determined on the basis of yield components (number of stems m^{-}, number of pods per stem, number of seeds per pod, thousand grain weight), and recalculated on the seed yield in kg ha^{-}. The results were analyzed by the analysis of variance of the two-factorial trial using SPSS software (1995). The significance of differences in mean values was tested by LSD test.

RESULTS AND DISCUSSION

Perennial forage legumes as nitrogen fixing plants are minimaly fertilized with nitrogen fertilizers, whose remains easily leach from the soil, causing contamination of groundwater and local water course (Janzen and McGinn, 1991). The pursuit of environmental protection, given the current climate change, indicates on the necessity of using measures such as pre-sowing inoculation of the birdsfoot trefoil seed.

Table 1. The effect of seed inoculation (Ø - control, *R_1– Mesorhizobium loti* (strain 608) *i* R_2– *Mesorhizobium loti* (strain Z)) on the seed yield and yield components of birdsfoot trefoil cultivars

	Treatmants	Stem number per m^{-}	Pod number per infleorence	Seed number per pod	Thousand seed weight(g)	Seed yield(kg ha^{-})
Cultivars	Zora	353. 3 b	13. 02	24. 03	1. 25	1364
	K-37	399. 6 a	11. 89	23. 18	1. 16	1266
	Rocco	373. 7 ab	11. 84	22. 71	1. 22	1236
Mesorhizobium	Ø	346. 2 b	12. 61	23. 62	1. 16 b	1186 b
	R_1	370. 9 b	12. 91	23. 54	1. 27 a	1422 a
	R_2	409. 4 a	11. 23	22. 76	1. 20 ab	1257 ab
Zora	Ø	300 c	12. 87	24. 5	1. 29 a	1221 bc
	R_1	335 bc	13. 9	25. 1	1. 33 a	1561 a
	R_2	425 a	12. 3	22. 5	1. 12 bc	1311 ab
K-37	Ø	427. 7 a	12. 2	24. 9	1. 11 bc	1418 ab
	R_1	362. 7 abc	12. 07	23. 3	1. 12 bc	1136 bc
	R_2	408. 3 ab	11. 4	21. 27	1. 25 ab	1243 bc
Rocco	Ø	311 c	12. 77	21. 5	1. 06 c	919 c
	R_1	415 a	12. 77	22. 2	1. 34 a	1571 a
	R_2	395 ab	10. 0	24. 5	1. 24 ab	1218 bc
ANOVA	Cultivars	★	ns	ns	ns	ns
0, 05	Mesorhizobium	★	ns	ns	★	★
	Cultivars x Mesorhizobium	★	ns	ns	★★	★★
CV (%)		11, 5	9, 7	9, 4	6, 9	14

The values denoted with different small letters within columns are significantly different at (P<0. 05) in accordance with the LSD test; *- F test significant at p<0. 05; **- F test significant at p<0. 01; ns - F test non-significant; CV - coefficient of variation.

In our experiment, a significant positive impact of pre-sowing seed inoculation on the number of stems m^{-} compared to the control treatment was recorded in treatment R_2 with cultivar Zora and in treatments R_1 and R_2 with cultivar Rocco (Table 1). The cultivar K-37 in control treatment had a significantly higher number of stems m^{-} as compared to the other cultivars of birdsfoot trefoil.

Irrespective of the pre-sowing seed inoculation, the cultivars did not significantly differ between themselves in terms of the number of pods per stem and the number of seeds per pod. The cultivars in average had 12. 2 pods per stem and 23. 3 seeds per pod. McGraw et al. (1986a) showed largely influence of number of inflorescences per stem as a component of yield on seed yield of birdsfoot trefoil. The pre-sowing seed inoculation of birdsfoot trefoil with bacteria *M. loti*, had no significant impact on the number of pods per stem and the number of seeds per pod regardless of the cultivars.

In our experiment the thousand seed weight ranged in average from 1. 16 in cultivar K-37 to 1. 25 in cultivar Zora. Grant (1967) states that in one gram is about 750 to 800 seeds of birdsfoot trefoil, or the mass of thousand seeds is about 1. 3 g. Pre-sowing seed treatment with bacteria *M. loti* positively influenced the thousand seeds weight only in the cultivar Rocco.

According to Turkington and Franco (1980) and Gullien (2007), the potential seed yield of birdsfoot trefoil is estimated at over 1200 kg ha^{-}. In our research in the second growth in the second year of cultivation, the average potential seed yield of birdsfoot trefoil of 1289 kg ha^{-} was achieved. According to McGraw et al. (1986a), the average harvest seed yield of birdsfoot trefoil varies from 50-175 kg ha^{-}, or about 100 kg ha^{-} of seed. Seaney and Henson (1970) point out that seed yields of birdsfoot trefoil vary from 50-560 kg ha^{-}. Seed yields of birdsfoot trefoil in USA range between 50 and 170 kg ha^{-} (Fairey and Smith, 1999), in Uruguay between 120 and 150 kg ha^{-} (Garcia et al., 1991; Artola, 2004) and Argentina between 25 and 150 kg ha^{-} (Mazzanti et al., 1988). According to Vojin et al. (2001), in agroecological conditions of the Republic of Srpska, in the area of Banja Luka, the seed yield of birdsfoot trefoil of 272 kg ha^{-} was achieved. In the Republic of Serbia the seed yield of birdsfoot trefoil varies from 100-280 kg ha^{-} (Vučković et al., 1997). At the full maturity trefoil pods easily burst and spill the seeds. The problem of bursting pods significantly limits the successful production of seeds (Fairey, 1994). According to Winch et al. (1985) losses at the seed harvest of birdsfoot trefoil are large, ranging up to 85% of potential yield. In our paper, shown the seed yield of birdsfoot trefoil is recalculated on the basis of the yield components, without taking into the account losses due to uneven ripening and bursting of pods. Uninoculated cultivar K-37 like the control treatment had a significantly higher yield than uninoculated cultivar Rocco, while there were no significant differences between other uninoculated cultivars. In general, seed inoculation with bacteria *M. loti*, had a positive effect on seed yield of birdsfoot trefoil. However, a significant positive effect of the pre-sowing seed inoculation on the yield was recorded only in the treatment R_1 of the cultivars Zora and Rocco. This is mainly due to the positive impact of inoculation on the seed yield components, primarily on the number of

stems m^{-} and thousand grain weight, thanks to the better repletion of the plants with nitrogen.

CONCLUSION

Inoculation of seed birdsfoot trefoil strains of *M. loti* significantly affected the seed yield components and seed yield. The both strains of bacteria of *Mesorhizobium loti* have made a positive impact on the aforementioned seed yield components of the birdsfoot trefoil cultivars. Inoculation of seeds had a significant effect on the increase of number of stems m^{-} in the cultivars Zora and Rocco and the thousand seed weight in the cultivar Rocco.

In terms of seed yield, the cultivars differed significantly among themselves in the control treatment. Cultivars Zora and Rocco had a higher seed yield only in the treatment of R_1 as compared to the control variant. The results indicate that the cultivar Rocco more positive reacted to the seeds inoculation with the bacteria *Mesorhizobium loti* as compared to the other cultivars and that thelowest worst reaction had the cultivar K-37. This further indicates that between the cultivars of birdsfoot trefoil there were differences in preferences for symbiotic relationships with different strains of bacteria. This tendency is conditioned by the degree of compatibility between the different cultivars and strains of rizobial bacteria. The results of ANOVA showed that the mutual interaction cultivar x strain, significantly affected the number of stems per m^{-} and the number of pods, and a very significant on the thousand seed weight and potential seed yield.

Therefore, further work should be focuced on finding the most appropriate combination of cultivars, species and strains of bacteria, in order to improve the production to the maximum in the given circumstances.

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