EFFECTS OF BACTERIAL INOCULATION (*Rhizobium leguminosarum* L.) ON NODULATION, YIELD AND AGRONOMICAL CHARACTERISTICS OF PEA (*Pisum sativum* L.)

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Rezumat

Cercetările prezentate în articol au fost realizate în regiunea Bursa, Turcia, în sezonul de vegetaţie 2015. În investigaţie au fost incluse soiurile Utrillo şi Bolero. S-a constatat că efectul inoculării bacteriene asupra numărului de nodozităţi şi asupra masei nodozităţilor uscate a fost semnificativ la începutul etapei de înflorire şi la faza de înflorire completă, în timp ce producţia de boabe şi greutatea a 1000 de boabe practic nu a fost influenţată. Parametrii menţionaţi au variat esenţial în funcţie de soi. Astfel, numărul de nododozităţi a variat între 14,5 şi 30,9, masa a 1000 de boabe a fost cuprinsă între 156,4 şi 276,4 g, iar randamentul acestora a constituit 1667-1909 kg ha^{-1}.

Cuvinte-cheie: mazare, inoculare bacteriană, nodozităţi, simbioză, randament.

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Introduction

Grain legumes are an important component of future food supplies in the World. They have played a crucial role in agricultural production throughout history. Grain legumes have been the most important vegetable protein sources used in plant nutrition so far. They are especially important for the people of underdeveloped and developing countries in which animal protein sources are limited and expensive.

Legumes have an important role for sustainable agriculture and food security too. Legumes may reduce nitrate leaching risk which leads to pollution of ground water. An increase in NO_3-N content of ground water has been widely reported in areas where heavy application of fertilizers and irrigated cropping systems take place. When fertilizer - N is expensive or unavailable, crop production systems depend on the N fixed by legumes to maintain the N cycle at the sustained productive level [5]. Moreover, they act in the maintenance and improvement of soil fertility by leading to the movement of plant nutrients in the lower layers of soil to the upper parts, thanks to their deep root system. Therefore, the legumes that can be grown in a given ecology should take part in crop rotation in sustainable agriculture. The nitrogen which is one of the reasons of green revolution is the main factor that limits the productivity of plants. It is given to plants as a natural or artificial fertilizer. In recent years, nitrogen provided by atmospheric happening and by organism which absorbs nitrogen in air in recent years because of the harms given to the environment and the food items by fertilizer. A lot of researches have been done intensively on the source of this nitrogen. Nitrogen gained by this way in a ready and economic sources that both legumes and non-legume plants can consume [8, 9].

Legumes have the capability of meeting their nitrogen requirements by converting the nitrogen taken by Rhizobium spp. bacteria from the air to a usable form for themselves, which is a type of symbiosis. They are able to supply 50-200 kg ha^{-1} pure nitrogen to the soil under suitable conditions after they are harvested [1, 2, 14]. Many grain legumes are efficient at N fixation. Variability affecting quantity of N fixed include not only legume species and cultivar, but also such factors as soil type and texture, pH, soil nitrate-N level, temperature and water regimes, availability of other nutrients, and crop (especially harvest) management [7].

To enhance legume nodulation and nitrogen fixation, the introduction of bacterial inoculants to agricultural fields has been a common practice approximately for 100 years. Whenever the specific rhizobia are absent, inoculation readily enhances plant growth and yield [15, 16].

Pea inoculated with the appropriate strain of Rhizobium bacteria is able to meet a large portion of its nitrogen requirement from air in the soil. Pea can supply just about 50-110 kg ha^{-1} pure nitrogen, if the condition of rhizobium symbiotically life is effective in the environments of pea [2]. Peas can meet their N needs between 30-80% through biological fixation [6]. Because of this, the pea seed or the pea-sown soil must be inoculated. If there is active native *Rhizobium leguminosorum* in the soil, there will be a competition between this native *Rhizobium leguminosorum* and inoculated *Rhizobium leguminosorum*, because of this, an efficient symbiosis will not be observed between pea and *Rhizobium leguminosorum*.

This study was conducted in order to analyze the effect of *Rhizobium leguminosorum* inoculation on seed yield, some yield components, nodule numbers and nodule dry weight.

Material and methods

Field experiment was carried out in the field of Mustafakemalpasa, Bursa located in southern Marmara Region of Turkey, in 2015 growing season. Total rainfall of growing season was 266.2 mm and mean air temperature was 15.5^{o}C. Watermelon was grown in that field the previous year, and no legumes were grown there within the last decade. The experimental field is 25 m above of the sea level. The soil was lightly alkaline-neutral lime with harmless salinity.

The experiment was designed in randomized complete block design with five replicates. Utrillo and Bolero cultivars which did not have any chemical treatment were used in the research. Seeds of both cultivars which were not pesticide-contaminated were either (1) inoculated by *Rhizobium leguminosarum* bacteria culture, or (2) sown without inoculation (control).

The *Rhizobium leguminosarum* bacteria culture was inoculated on the surface of seeds with water without chlorine, or any chemical residue (100 kg seeds, 200 g inoculation material) just before sowing. 150 kg ha^{-1} Diammonium phosphate (DAP) composed fertilizer (18-46%) was applied prior to sowing in the soil with disc-harrow (just about 32 Kg N and 69 kg ha^{-1} P_2O_5). No pesticide was applied to the seed and soil at sowing. There was not any irrigation for the growing season. Planting was made on 18 March 2015. The plot size was 10 m x 1.6 m. Each plot had four rows. The row and in-row spacing were 40 and 5 cm, respectively. Plot area was 16 m^2. At harvest time, each block had two rows at the beginning and at the end of the block for protection which were removed before harvest. Hand hoeing control was done twice after rains (at vegetative stage and the beginning of flowering) by hand. Plots were harvested by hand at the end of June when the plants were 90% mature.

The data were analyzed according to randomized complete block design. The obtained data were statically tested using JUMP statistical programme. The significance of differences was determined with LSD test at 0.05 probability level.

Results

Significant differences were found between cultivars at 0.01 probability level in terms of all the characters measured. Nodule number (the beginning of flowering and full bloom period) and nodule weight (full bloom period) were significantly affected by rhizobium inoculation. Analysis of variance showed that grain yield and 1000 seed weight were not significantly affected by rhizobium inoculation (Table 1).

Table 1. Degrees of freedom, mean squares and coefficients of variation determined for nodule numbers, nodule weights, 1000 seed weights and seed yields of two different pea cultivars subjected to bacterial inoculation.

Source of variation	Degree of freedom	Number of nodules at the onset of flowering (nodules/plant)	Number of nodules at full-bloom stage (nodules/plant)	Nodule weight at full-bloom stage (mg)	1000 seed weight (g)	Seed yield (kg ha^{-1})
Replicates	4	8.77425	4.707	0.001725	50.725	8095.725
Cultivar	1	1033.9220**	856.74050**	0.30890**	69974.45**	243321.80**
Inoculation	1	46.2080*	54.78050**	0.00744**	14.45	2289.80
Cultivar * Inoculation	1	7.2000	1.30050	0.000009	1.25	561.8
Error	12	6.068	3.72	. 00012916	44.725	8215.09
General	19	62.90	51.38	0.01782894	3722.765	19849.463
Coefficient of variation		**11.39**	**8.6**	**5.1**	**3.1**	**5.1**

**** 0.05 (Statistically significant at 0.05 probability level)***
***** 0.01 (Statistically significant at 0.01 probability level)***

The effect of rhizobium inoculation on the number of nodules formed on plant roots at the onset of flowering was found significant, and it was observed that more nodules formed on the roots of plants grown on rhizobium-inoculated plots (22.6 nodules), compared with non-inoculated ones (20.1 nodules). During this period, number of nodules on the plants of cv. Bolero was found as 28.8 number/plant, which is much higher than cv. Utrillo (13.4 number/plant) (Table 2 and 3).

Table 2. Values of nodule number and weight recorded during the onset of flowering and full bloom stages for two different pea cultivars subjected to bacterial inoculation.

Cultivars	Nodule number (nodules/plant)						Nodule weight (mg/plant)		
	Onset of flowering		Mean of cultivars	Full bloom		Mean of cultivars	Full bloom		Means of cultivar
	inocu-lated	non-inocu-lated		inocu-lated	non-inocu-lated		inocu-lated	non-inocu-lated	
Utrillo	15.36	13.52	13.4 b	17.32	14.52	15.9 b	18.72	17.54	18.1 b
Borello	30.94	26.70	28.8 a	30.92	27.10	29.0 a	26.62	25.36	25.9 a
Means	23.2 a	20.1 b		24.1 a	20.8 b		22,7 a	21.5 b	

Rhizobium inoculation effect on nodules number at full bloom was found as significant. Number of nodules on the plants in inoculated plots (24.1 number/plant) were higher compared with non-inoculated ones (20.8 number/plant). During this stage, more nodules were formed on cv. Bolero (29.0 nodules per plant), compared with cv. Utrillo (15.9 nodules per plant) (Table 2). Our findings are parallel to the results of Mahdi (1992) [12], Tahir et al. (2009) [17], Bejandi et al. (2012) [3] and Kumar et al. (2014) [13] who reported that nodule numbers increased by inoculation.

Table 3. Values of 1000 seed weight and seed yield recorded during the onset of flowering and full bloom stages for two different pea cultivars subjected to bacterial inoculation.

Cultivars	1000 seed weight (g)			Seed yield based on 10% seed moisture content (kg/ha)		
	After drying		Means of cultivar	After drying		Means of cultivar
	inoculated	non-inoculated		inoculated	non-inoculated	
Utrillo	276.4	274.2	275.3 a	1909	1877	1893.2 a
Borello	157.6	156.4	157.0 b	1678	1667	1672.6 b
Means	217.0	215.3		1793	1772	

Effect of *Rhizobium* inoculation on dry nodule weight per plant was found statistically significant, and dry nodule weight regarding the plants on rhizobium-inoculated plots was found higher with 22.7 mg per plant, compared with the plants on non-inoculated plots (21.5 mg per plant). Plants of cv Bolero had higher nodule weight (25.9 mg/plant), compared with cv. Utrillo (18.1 mg/plant) (Table 2). Our findings are in accordance with those of Ngeno et al. (2012) [13].

Effect of bacterial inoculation on 1000 grain weight was found insignificant statistically. 1000 seed weight of cv Utrillo (275.3 g) was higher than that of cv Bolero (157.0 g). Our findings are in accordance with the results of Kumar et al. (2014) [11] and Ngeno et al. (2012) [13]. Effect of bacterial inoculation on seed yield was also found insignificant statistically. More seed yield was obtained from cv Utrillo (1893.2 kg ha^{-1}) compared with cv Bolero (1672.6 kg ha^{-1}) (Table 2). The results were similar to those obtained by Kumar et al. (2014) [11] and Ngeno et al. (2012) [13].

Conclusion

All nodules formed at plant root zone are not effective nodules. Assuming that pink and larger nodules are more effective in nitrogen fixation, it may be suggested that they will not be effective on seed yield. Although bacterial inoculation is effective on the number and dry weight of nodules, it may not lead to an effective N fixation. On the other hand, it is rather difficult to determine to which extend the inoculation material affected the nitrogen fixation when the inoculation treatments were carried out field condition (Karasu et al., 2009, Cebel and Altuntas, 1992), since nodule formation and nitrogen fixation are greatly affected by environmental factors (Azkan, 2002).

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