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Evaluating the effects of different planting densities and inoculation with Rhizobium bacteria on morphological characteristics and yield of faba bean (cv.Barakat)

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Abstract

Among legumes, Faba beans due to having proteins and essential amino acids and the ability of nitrogen fixation and symbiosis with Rhizobium bacteria, have high importance in terms of food and environmental effects. Growth and yield of Faba beans, like other crops, are functions of environmental factors and plant genotype. The experiment was conducted in the field area of300sq.m.ina completely randomized block design with split plots (split-plot) in three replications in the fall of 2009. Planting density, as the main factor of the test, has three levels: 1) A_1: density of 100,000 plants per hectare (20 cm row plant spacing), 2) A_2: density of 50,000 plants per hectare (40 cm row plant spacing), 3) A_3: density of 25,000 plants per hectare (60 cm row plant spacing). Also the sub-factor of the experiment has 2 levels: 1) B1: seed not inoculated with bacteria, 2) B2: seed inoculated with bacteria. The results show that an increase in bush density causes a significant increase in total dry matter production per unit area, but in contrast, total dry matter per plant reduced due to high competition. Also, it was observed that Fababean seed inoculation with bacteria before sowing had a positive and significant effect on bush height, grain weight, grain filling of pods, number of pods, length and width of pods, reduction of flowers` fall and traits associated with yield and yield components. The study of the interactive effect of planting density and bacteria on Faba bean indicated that bean bushes containing seeds with bacteria and spacing 20 cm between plants on the row (a1b2) showed best results in terms of yield and yield components, bush height, plant growth.

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Introduction

According to the FAO report, world population will reach to about 9.8 billion in 2050 (FAO, 2010). Population growth, food shortages and high food prices have caused the increase in the population of hungry people in the world. So to feed the world's growing population, it is necessary to increase food production (Yuksel and Memon, 2009). Faba bean`s ripe grains contain 23.4%-36.4% protein essential amino acids such as Lysine, Arginine, Methionine, Sparzhyn and plenty of minerals such as Phosphorus(P), Calcium(C), Iron (Fe), Magnesium(Mg) and Potassium(K) (Abdalla, 1979). Varieties of Faba beans with small grains grow in temperate regions, and varieties with medium to big grains grow in Mediterranean tropical and subtropical regions. Faba bean yield is estimated through calculation of the number of bushes per hectare, pods per plant, grains per pod and weight of 100 grains (Feredrick and Marshall, 1985) Maximum yield is achieved when all components are functioning at their maximum level, but due to competition, yield components have negative correlation with each other (Abdalla, 1979; Atsushi and Toshinori, 2006). In general, vegetative and reproductive stages in legumes overlap, for example, after the beginning of flowering in beans, pods begin to grow. Also differentiation of new leaves and flower buds on growing stems will continue for a long time. So pods (sink) and leaves (source) continue to grow simultaneously and as long as the capacity of the source does not satisfy sinks` need, competition arises between these two components. In general, growth and yield of crops are functions of environmental factors and plant genotype. Planting density is one of the environmental factors that can directly affect product yield. Plant spacing in Faba bean depends on cultivation goal. If the goal is to produce grains, planting space should be increased to increase the quantity and quality of product. If the goal is to produce green manure and livestock forage density will be further considered. Of course, the very high plant density increases competition within a species, reducing the number of nodes per bush, flowers per bush, grain weight and ultimately yield (Jones, 1992; John and Mahon, 1979).

In the low planting density, the amount of dry matter accumulated in pods, stems and leaves in the same number of days after planting is more than high planting density. Meanwhile, increase in the dry weight of pod is more than that in stem, leaf and with the onset of pod filling, the accumulation of materials in these organs rises due to the strength of the sink (Noroozi*et al.,* 2007). Feredrich (1985) and Abadi and Sedaghatpour (2006) reported that although decrease in bush density increased the number of nodes per bush, 100 grains weight, number of branches per bush, number of pods per bush, the grain`s dry matter, nitrogen, yield and number of grains per pod did not change significantly. Proper spacing between rows and between bushes in the rows determines the proper and usable growing space for each bush. Among the nutrients, plants need nitrogen more than other elements. Nowadays, due to the high cost of producing nitrogen fertilizer and environmental pollution caused by fertilizers, more attention has been focused on nitrogen fixation by different organisms. It is estimated that 15-18 million tons of biological nitrogen fixation is done annually, about two-thirds of which can be achieved through symbiotic biological fixation. Khosravi and Ramezanpour (2004) evaluated the effectiveness of several Rhizobium inoculants on the growth of Faba bean (cv. Barakat) and reported that seed inoculation with Rhizobium bacteria symbiotic with the roots of Faba bean (*Rhizobium leguminous*arumb. v. *viciae*)increased seed yield and weight of 100 grains between 35 to 69 percent compared to the control (not inoculated with bacteria). Also they noted that Faba bean acquire 79% of its required nitrogen through biological fixation, 20% from soil and only one percent from fertilizers.

Vargas*et al.* (2000) studied the bean plant and observed that this plant can achieve maximum performance and protein through seed inoculation with nitrogen-fixing bacteria and consumption of low amount of nitrogen as Starter (only 15 kilograms/ha urea).Bean varieties have different amounts of nitrogen fixation. Two varieties Saraziri and Barekat have higher dry matter yield and nitrogen fixation, compared with Shahi variety. Due to the multiple and sometimes contradictory results of different tests, it can be said that the effect of environmental factors on bean yield is yet a challenge. The present study was carried out in order to investigate the effects of planting density and seed inoculation with bacteria on bean yield and soil fertility in a given climate (Kashan).

Materials and methods

Location and experimental design

The experiment was conducted in the field area of 300 sq.m² in a completely randomized block design with split plots (split-plot) in three replications in Hasan Abad village located 5 km southwest of Kashan in the fall of 2009. Three planting density were evaluated in this study: 1) A1: density of 100,000 plants per hectare (20 cm row plant spacing), 2) A2: density of 50,000 plants per hectare (40 cm row plant spacing), 3) A3: density of 25,000 plants per hectare (60 cm row plant spacing). In order to Seed inoculation with Rhizobium bacteria, about 3 ml of bacteria processed and reproduced with bacterial inoculum (prepared by the Research Center for Agriculture and Natural Resources, Gorgan) were mixed with 5 kg of bean seeds by stirring thoroughly for 5 minutes and then planted immediately after the inoculation.

Crop management

All land and planting bed preparation measures, including plowing, disk leveler and plot layout drawing with dimensions of 2/5 × 4 m were fully carried out. Blocks were 4 meters apart in the land. There were 6 plots in each block with a distance of 1 m from each other and 5 growing lines in each plot with 4 m length. Planting rows of each plot were 50 cm apart. The first irrigation after planting was performed and then the farm was not irrigated until germination (15 days). After 12-15 days all seeds in different plots germinated.

Traits

In this experiment, the number of days from planting to emergence, stem elongation, budding, flowering, pod formation, pod and grain filling and final maturation of grain and pods were accurately measured. Also important morphological traits in the bean plant including height (every 15 days), length of main stem, number of branches, length, width and weight of green, ripe and dry pods, number of pods per bush, number and weight of grains per pod and weight of grains per plant were measured. Final grain yield, biological yield and harvest index were also evaluated in the final maturity stage.

Statistical analyses

Analysis of variance was performed for all traits by SAS (9.1) software. Means were separated by application of Duncan's test when the F test proved significant at $P = 0.05$.

Results and discussion

Plant density (plant spacing in row)

The results show that increasing density (decreasing the space in the plant row) causes a significant increase in total dry matter production per unit area. But in contrast, total dry matter per plant decreases, due to high competition. Also with increasing the density of total dry matter produced, grain yield and number of pods per bush was significantly increased. This can be attributed to the compensation for the reduction in dry matter of single plant as a result of increasing the number of plants per unit area. On the other hand, increasing the plant density reduces the number of branches per plant and harvest index. One of the main reasons for this decline could be the increase in competition and decrease of the space required for growth and development of plant organs (Gisele *et al.*, 2003).

Weight of 100 grains is considered as a major factor in determining the Faba bean yield. The evaluation of this characteristic in the control plots (with seeds not inoculated with bacteria), and comparisons between the cultures of different densities show that although plant density in A1 (plots with 20 cm row plant spacing) was higher and it was expected to have lower grain weight and yield, the weight of 100 grains in A1 was more than A2 and in A2 was more than A3. But in contrast, in plots with seeds inoculated with bacteria, the weight of 100 grains in A2 (plots with 40 cm row plant spacing) was more than A1 (plots with 20 cm row plant spacing) and in A3 (plots with 60 cm row plant spacing) was the least (Table 1).

Table 1. Weight of 100 grains in Different planting density and in both inoculated and non-inoculated with bacteria.

Plant density Level of inoculation	A1 (plots with 20 cm row plant spacing)	A2 (plots with 40 cm row plant spacing)	A3 (plots with 60 cm row plant spacing)
B1 (seed not inoculated with bacteria)	157	153	153
B2 (seed inoculated with bacteria)	165	165	156

Table 2. Correlation between final grain yield, biological yield, harvest index and morphological characteristics (plant height, weight of 100 grains, number of grains per pod, length and width of pod, number of pods per plant and Ripe seed pod of a plant).

x23	x22	x21	x20	x19	x18	x17	x16	x15	x14	x13	x12	x11	x10	x9	x8	x7	x6	x5	x4	x3	x2	x1	
																						1.00	x1
																					1.00	.854**	x2
																				1.00	0.36	.533*	x3
																			1.00	.690**	0.29	.565*	x4
																		1.00	.697**	.577*	0.47	.548*	x5
																	1.00	.792**	.585*	.535*	0.31	0.38	x6
																1.00	.481*	.697**	.831**	.653**	0.09	0.36	x7
															1.00	.742**	.669**	.759**	.755**	.739**	0.40	.513*	x8
														1.00	.600**	.790**	0.42	.608**	.510*	0.33	-0.20	0.07	x9
													1.00	.576*	.636**	.756**	.621**	.646**	.814**	.682**	0.19	.531*	x10
												1.00	.631**	.578*	.549*	.570*	0.46	.646**	.590**	0.41	0.13	0.37	x11
											1.00	0.23	.486*	0.32	.534*	.537*	0.45	0.43	0.42	.746**	0.24	0.44	x12
										1.00	.717**	0.03	0.19	0.30	0.25	0.27	0.19	0.12	0.10	0.47	-0.19	0.06	x13
									1.00	.864**	.820**	0.21	0.26	0.29	0.36	0.41	0.29	0.26	0.22	.639**	0.09	0.29	x14
								1.00	1.000**	.864**	.820**	0.21	0.26	0.29	0.36	0.41	0.29	0.26	0.22	.639**	0.09	0.29	x15
							1.00	.805**	.805**	.787**	.860**	0.10	0.33	0.30	0.45	0.41	0.21	0.24	0.27	.690**	0.12	0.32	x16
						1.00	.561*	.709**	.709**	.493*	.757**	0.07	0.38	0.11	0.37	0.37	.618**	0.47	0.33	.647**	0.35	0.36	x17
					1.00	0.41	.563*	.767**	.767**	.792**	.620**	0.24	0.31	.559*	0.42	.606**	0.23	0.25	0.40	0.43	-0.19	0.10	x18
				1.00	0.46	0.33	0.38	.551*	.551*	0.36	.597**	0.17	0.17	0.10	0.32	0.22	0.15	0.09	0.16	0.35	0.31	0.46	x19
			1.00	0.05	-0.30	0.27	-0.15	-0.16	-0.16	-0.41	-0.01	0.15	0.14	-0.08	0.27	0.13	0.31	0.39	0.28	0.24	.558*	0.37	x20
		1.00	-0.21	0.11	.695**	0.34	.503*	0.45	0.45	.589*	.519*	0.14	0.46	.513*	.677**	.666**	0.46	.511*	.633**	.538*	0.05	0.27	x21
	1.00	.574*	-0.03	.519*	.813**	.651**	.518*	.767**	.767**	.627**	.695**	0.19	0.21	0.34	.503*	.518*	0.35	0.36	0.31	0.45	0.17	0.23	x22
1.00	0.41	.715**	-0.05	0.16	.507*	0.34	.518*	.489*	.489*	.558*	.490*	0.16	0.36	0.34	.625**	0.43	0.31	0.44	.484*	.684**	0.20	0.35	x23

X1: Height of plant 5 month from emergence (cm), x2: Height of plant 4.5 month from emergence (cm), x3: Height of plant 4 month from emergence (cm), X4: Height of plant 3.5 month from emergence (cm), x5: Height of plant 3 month from emergence (cm), x6: Height of plant 75 days from emergence (cm), x7: Height of plant 60 days from emergence (cm), x8: Height of plant 45 days from emergence (cm), x9: Height of plant 30 days from emergence (cm), x10: Height of plant 15 days from emergence (cm), x11: Height of plant 15 days from planting to emergence (cm), x12: harvest index, x13: biological yield (g/m^2), x14: final grain yield (kg/ha), x15: final grain yield (kg/10 m^2), x16: final grain yield per plant (g), x17: Weight of 100 grains (g), x18: Weight of green (g/10m^2), x19: Number of grain per pod, x20: Width of pod (mm), x21: Length of pod (mm), x22: Number of pod per plant, x23: Weight of ripe and dry pod per plant (g).

Rhizobium bacteria

The results show that inoculation of Faba bean`s seed with bacteria before planting has a positive and significant effect on the grain weight, pod filling, number of pods, length and width of pods, reducing the flower falling and all traits associated with yield and yield components. However, in this experiment seed inoculation with bacteria didn`t have significant effect on the number of grains per pod and the final yield in 10 square meters. Genetic factors presumably exert more control, than environmental factors, over these two characteristics. Plant`s height is a very important morphological characteristic. Increasing the plants height can be an advantage, in terms of competition with other plants in a plant community. In addition, one of the results of increase in plant`s height is the formation of new and young leaves at the top of the canopy. Therefore, the most effective leaves locate in the best position in terms of photosynthesis (Martin and Downie, 2008). In this experiment, after measuring the plant`s height from the time of emergence to the end of pod filling stage and the beginning of grain filling stage(150 days after emergence) it was observed that bacteria had a very effective and positive role in increasing the plant`s height and green area of the canopy. Furthermore, bacteria had Avery effective and positive role in increasing and improvement of the other morphological characteristics such as pod filling, texture, length, width and weight of grains and weight of grains in plant. Moreover, tillering and growth continued to after harvesting the ripe pods in the plots containing seeds inoculated with bacteria but in control plots (plots with seeds not inoculated with bacteria) no such thing was observed.

Table 3. analysis of variance of plant Analysis of variance

Variations	grade freedom	X22	X21	X20	X19	X18	X17	X16	X15	X14	X13	X12
		Number of pods per plant	Pod length (mm)	Pod width)mm)	Number of grain in pod (g)	Average of final dry matter	100grains weight	final yield of seed per bush(g)	Final seed yield in 10m3(kg)	Final seed yield in hc(kg)	bush biologicyield in1m3(g)	Harvest index
Replication R	2	1/287	1509/291 *	5/669 *	0/234 n.s	1574296/560 n.s	65/722 *	487/593 *	1/942 n.s	194228/72 n.s	246289/858 **	24/132 *
Planting density(A)	2	0/067 **	661/274 **	6/647 *	0/021 *	28/761 **	55/056 *	368/732 *	37886 n.s	378/860 n.s	238/749 **	9/700 **
Ea	4	0/117	398/621	2/903	0/192	24/713	15/389	51/828	3974	39/740	80/031	2/788
main factor(B)	1	1/176 **	1270/080 **	15/039 **	0/269 n.s	93/781 *	288 **	451/401 *	233928 n.s	2339/280 n.s	48/269 *	55/479**
(A × B)	2	0/004 n.s	66/185 n.s	19/845 n.s	0/051 n.s	57/362 n.s	26/167 n.s	26/674 n.s	20088 n.s	200/880 n.s	1/175 *	5/806 n.s
Eb	6	0/083	64/133	0/727	0/078	33/358	11/944	52/350	283518	2835/180	4/692	1/989
c.v variation coeeficient		0/06	0/09	0/10	0/05	0/01	0/04	0/14	0/39	0/39	0/28	0/14

Reciprocal effect of density and bacteria

Faba bean plants in plots containing seeds inoculated with bacteria and 20cm spacing between plants on the row (A1B2) showed best results in terms of yield and yield components, plant height and growth, plant resistance to pests, diseases, weeds and nutrients deficiencies. The final grain yield in plots A1B1, A1B2, A2B1, A2B2, A3B1 and A3B2 were evaluated as 4.5, 5.3, 3.8, 4.3, 3.7 and 3.9, respectively. Evaluation of the relationship between plant height (from 15 to 105 days after germination)and planting density showed that due to competition between plants for water and nutrients in the a1 plots there was a significant positive correlation($p<0.01$, $r = 0.814$) between the density and plant height (growth). Evaluation of the plant height in the period of 4/5 to 5 months after germination showed that maximum plants` height was in plots a1 and A2 (spacing plants 20 cm and 40 cm) and plant height in plots with bacteria(A1B2 and A2B2) was more than that in control plots (A1B1 and A2B1). This indicates the positive role of bacteria in supplying nitrogen as an effective element needed in the growth and photosynthesis of plants. Moderate increase in plant height, if accompanied by an increase in stem diameter, is an advantage in yield improvement due to increasing leaf area index and its better distribution in plant`s canopy. Also it has a positive effect on controlling weeds and reducing the evaporation of surface water in between rows and plants in the plots in all stages of growth as well as mechanized harvesting. Harvest index of legumes can be increased in a given area (0.0%-30%). In this experiment, harvest index in plots a1b2 and a2b2 was observed up to 28%.Given the significant correlation between harvest index and plant height 4 months after germination, one reason for the increased harvest index in these two plots can be moderate increase in plant height. Despite the positive relationship between final grain yield and harvest index, biological yield and harvest index showed a negative relationship (Table 2). Thus, plots with higher harvest index, had less biological yield. In this experiment, the plots A1B2 and A2B2 having the highest harvest index, had a low level of biological yield (mean final dry weight of plant shoots). In general, there are reverse relationships between yield components, so that an increase in one component leads to a decrease in other (s) component (s). Reducing the number of grains per plant causes an increase in grain weight, so the grain weight and economic performance increase. It also compensates for the loss of biological yield and finally, changes in grain yield are minimized. In this study, a positive concurrency was observed between harvest index, plant height (appropriate growth and the green area of plant), effect of Rhizobium bacteria on the supply of nutrients needed for plant growth (available nitrogen), compared to control plots, and appropriate distance between plants on the row that finally led to an increase in grain yield (10 m^2) in plots and grain yield per unit area. Moreover, the lowest correlation between harvest index, biological yield and plant height can be justified according to the low height of plants in the third density (A3), especially in plots without bacteria. In this experiment, a negative correlation was observed between harvest index and pod width($r=-0.01$). This negative relationship is likely to indicate that the pod width is mostly under the control of plant genotype and favorable environmental conditions don`t affect harvest index and final grain yield. Unlike the pod width, the most important characteristics influencing yield including the number of grains per pod, number of pods per plant, grain weight and grain weight per pod had positive and significant correlation with harvest index (Table2).

Yield and yield components

Yield components consist of number of bush, pods in bush, Length and width of pod, number of seed in pod and seed (grain) weight. The results showed that there is a significant difference among yield components, seed final yield per plant in plots a_1 b_2 involving 100 seed weight, pod width, pods with mature seeds weight, number of seed in pod (p=%5). In bush density and bacterium, harvest index, biologic yield, final dry matter average, length of pod, number of pods per plant and seed final yield a significant difference was achieved (p=%1). Based on reciprocal effect in traits like number of seeds in pod and bush, average off final dry matter, weight of pods with dried matured seed, length and width of pod, 100 seed weight, seed final yield per plant and harvest index the reciprocal effect of density and bush was not significant but it was significant in bush biologic yield in m^2, seed final yield by p=%5 between density and bacterium and by p=%1 between density and replication (Table3).

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