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Effect Inoculation of Soybean Cultivars with bacteria *Rhizobium japonicum* in Sistan

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ABSTRACT: This study is performed in order to investigate the inoculation effects of soybean cultivars with *Rhizobium japonicum* bacteria on yield and yield performance of these cultivars. The experiment was in the form of randomized complete block design split plots which conducted at Agricultural and Natural Resource Center of Zahak with three replication in 2012-2013. Experimental levels were consisted of non-inoculated and inoculated with rhizobium bacteria as the main factor, and the cultivars (varieties) factor with six level included M7, Sahar, Williams, M9, Zan, L17 which distributed in subplots randomly. The investigated quantitative and qualitative characters in this study consisted of plant height, number of pods per plant, number of seeds per pod, grain weight, biological yield, harvest index, raw protein, raw fat; and phonological traits included flowering onset, flowering period, and physiological maturity. The comparison between the obtained results and the results of control treatment (non-inoculated) and also the results of other studies illustrated the optimized and desired effect of inoculation on the traits such as yield and raw fat rate, so that in non-inoculation treatment grain yield decreased (the obtained yield was 1058.6kg/ha). Inoculation treatment with an increase of 9.91 percent, improved the grain yield which reached to the amount of 1190.4kg/ha. Among the experimental varieties, M7 with an average of 1467.5kg/ha had the highest grain yield, while the least amount of yield belonged to Zan and Sahar with the average of 861.2kg/ha. Interaction between inoculation and cultivar on all of the characters was not significant. Based on the results of this study, we conclude that the use of inoculated M7 variety can be introduced as the recommended cultivar among other varieties.

Key words: Soybean, Inoculation, yield, variety (cultivar)

INTRODUCTION

Among the plant oils, soy has special features. It has a wide and varied range of applications. Soybean oil is one of the main components of edible oil market. And to feed of many people is consumed especially as margarine and hydrogenated fats. Soy is a valuable and strategic plant with 35 to 45 percent and 18 to 22 percent of the oil in the seed. In addition to the various uses, vegetable oils in the diet, in the industry is also made of it, various materials. Soybean oil contains unsaturated fatty acids such as oleic acid, linoleic acid, linolenic acid and This unsaturated fatty acids, are very important in terms of supplying vitamin and maintaining health of humans. The use of bacteria at planting soybeans instead of nitrogen fertilizer adds to its importance. Nitrogen fixation by Rhizobium bacteria by reducing the use of nitrogenous fertilizers and related costs prevent from nitrate pollution resulted from fertilizer. Although biological nitrogen fixation systems is a long-term process in agricultural, so as a sustainable source of energy is important in agriculture. And has reduced from the use of needed nitrogen fertilizers for food production in recent decades. Soil microbiology experts always try to focus on the isolation and identification of Rhizobium strains of soybean symbiotic that have appropriate efficiency in Nitrogen fixation. While agricultural scientists are seeking to find the ideal potential growth in terms of potential of soybean performance yield and growth of various types (Medium, early and late) in different environmental conditions and consistent, with the change in factors such as planting date, planting pattern, and so on.

But it is more correct to introduce for each climate zone, appropriate soybean varieties and efficient strains of tested bacterial and the best combination of bacteria. In overall, the number of root nodules and their distribution patterns soybean roots depends on the varieties. In each figure is different with others. Among the plant oils, soy has special features. It has a wide and varied range of applications. Soybean oil is one of the main components of edible oil market. And to feed of many people is consumed especially as margarine and hydrogenated fats. Soy is a valuable and strategic plant with 35 to 45 percent and 18 to 22 percent of the oil in the seed. In addition to the various uses, vegetable oils in the diet, in the industry is also made of it, various materials. Soybean oil contains unsaturated fatty acids such as oleic acid, linoleic acid, linolenic acid and this unsaturated fatty acids, are very important in terms of supplying vitamin and maintaining health of humans. The use of bacteria at planting soybeans instead of nitrogen fertilizer adds to its importance. Nitrogen fixation by Rhizobium bacteria by reducing the use of nitrogenous fertilizers and related costs prevent from nitrate pollution resulted from fertilizers. Although biological nitrogen fixation systems is a long-term process in agricultural, so as a sustainable source of energy is important in agriculture. And has reduced from the use of needed nitrogen fertilizers for food production in recent decades. Soil microbiology experts always try to focus on the isolation and identification of Rhizobium strains of soybean symbiotic that have appropriate efficiency in Nitrogen fixation. While agricultural scientists are seeking to find the ideal potential growth in terms of potential of soybean performance yield and growth of various types (Medium, early and late) in different environmental conditions and consistent, with the change in factors such as planting date, planting pattern, and so on. But it is more correct to introduce for each climate zone, appropriate soybean varieties and efficient strains of tested bacterial and the best combination of bacteria. in overall, the number of root nodules and their distribution patterns soybean roots depends on the varieties. There is different in each figure with others. In investigating the culture of Asa and colleagues, the performance of tested yield at 1% level showed significant differences. They have announced among the studied figure, limited number of cultivars had the most performance of grain and told that the reason of it is limited and unlimited growth type of cultivars. So limited growth of cultivars as a result of formation of more pod than the number of indeterminate growth produce increasing performance.

In the review of (Hungry and Bohrer, 2000), also reported significant differences among soybean cultivars on the performance of soybeans inoculated with different strains of *Rhizobium japonicum* bacteria. Also (Amin *et al* 2008), reported highest performance at the Sahar rather than the Williams. In study of Kazemi *et al.* (2006), In relation to the impact of yield inoculated with bacteria on performance and components of soybean performance, reported that inoculation leads to significant increase in the final performance of soybean. Increase of yield performance in the Inoculated condition, in average was 20% in compared to control (non-inoculated).Also, there are significant differences in terms of grain performance between varieties. They noted that the reason of it, is being late and high potential of line 20 than Williams. In some studies the ability to provide the needed nitrogen of soybean to 95% have been reported (Peoples *et al.*,2004).Given the importance of tend to sustainable farming systems and also the importance of soy in supplying the human food, Due to the potential for agricultural production in arid and semi-arid region and to promote its cultivation in Sistan region, This research is carried out with the aim of introducing the best figure of six studied figure in inoculation with *Rhizobium* bacteria.

MATERIAL AND METHODS

The research implemented in agriculture year 1391-1392 in agriculture research station and natural resources of city of Zahak, located in 25 kilometers of southeast of the city of Zabol. Soil texture of test place was clay loam class. Other soil properties were given in Table 1. Seeding rate based on the research recommendations of 75 thousand plants per hectare was used for both plants alike. The experiment was conducted as A split plot design and in terms of A randomized complete block design in three replications.

Table 1.Characteristics of the tested soil.

EC(ds/ms)	6.36
PH	8.12
Soil organic matter	0.39
Nitrogen	0.06
phosphorus	2.6
potassium	190

Experimental levels were consisted of non-inoculated and inoculated with rhizobium bacteria as the main factor, and the cultivars (varieties) factor with six level included M7, Sahar, Williams, M9, Zan, L17 which distributed in subplots randomly. In each plot there is four cultivation lines with a length of 5 meters with distance of rows 50 and on the rows 5 centimeters and the distance between the plot was one meter. Planting in accordance with local custom on date 91,12,26 has been done as apparatus by drill machine according to the map of test. In inoculated treatments with bacteria liquid of *Rhizobium japonicum* according to the recommendation of the Microbiology Department, the Institute of Soil and Water Research (producing bacteria), at first we put 80 gram seeds in the plastic bag, And then with syringe 10 mg bacteria liquid was added to the seed then mixed together to make seminal fluid of seeds absorption. For better adhesion of bacteria to seed, we used adhesive and protective solution of this Institute. The first irrigation was done on 1384/5/10 and other irrigated as needed using a dipping method was done. And during the preparation of the land, 12 kg phosphorus and 23 kg potassium, respectively, of fertilizer resource of ammonium phosphate and potassium sulphate were given to soil. Two procedures were used to calculate the final performance. Harvested seeds for 48 h at 70°C were dried in an oven and then weighed. Data from experiments using SAS software was analyzed. Comparison of means was done using Duncan's test at 5%.

RESULTS AND DISCUSSION

A. Height of bush

variance results showed that plant height, although in the inoculation treatment with bacteria *Rhizobium japonicum* was further than un inoculated treatment but it was very small and not significant. It was while, the height of soybean plant under the influence of soybean cultivars was significant at 1% level. So the maximum height plant of Zan was achieved with mean of 5 cm. M7, Sahar, Williams, M9, Zan, L17 were placed in the next category in one group. Primarily limited growth figures have less height, While figures of unlimited growth, were some relatively short legs and others were somewhat tall legs. After arriving to the flowering stage, continue their growth. in total, figures of indeterminate growth cause appropriate vegetative growth and will guarantee performance (Khajuienejad, 2009). The results of current research was different in relation to the effect of inoculation on increasing height of plant, with many results of researchers in investigation on soybean varieties by strains of bacteria *Rhizobium japonicum.* Because they have pointed out to significant effect of bacteria in inoculation and their role in plant height. So we can refer to tests of Chandrasekar *et al* 2005 that in that experiments, is reported increasing of height of millet on inoculation with *Azotobacter* and *Azospirillum.* In experiments of (Akbari*et al*. 2010) to investigate the effects of inoculated seeds of Soybean cultivars with different strains of bacteria, the results has shown that bacteria have significant effect on plant height. In this experiment, soybean cultivars, in terms of plant height were different. Plant height, in investigating soybean cultivars by Asa *et al.* (2006) showed significant differences at probability level of 1 percent. They said that among the studied cultivars, cultivars of the mother, L17, Clark and 0,32 have the maximum of height Zan, Sahar have the minimum height. Hadi and et al stated that the plants of cultivar seeds Williams inoculated with soybean inoculums made 12 and 10%, respectively, greater stem height than the other cultivars. They also announced that, Since on the roots of plants from cultivar seeds Williams was made more nodes, has provided more nitrogen to the plant and has increased the length of cells and height of stem in comparison to other treatments.

B. The number of pod in plants

The results show that the number of pods in plants at the 5% level were at effect of inoculated treatment with bacteria *Rhizobium japonicum*. The highest number of pods achieved in plants of inoculated treatment with mean value of 54.11. That shows about 12.73 increase compared to non-inoculated treatment. The obtained results in relation to the number of pods in plant impressed with inoculation is correspond to the results of Kazemi *et al* 2006. Comparison of means in terms of inoculation with bacteria and non-inoculated with bacteria in their study showed that seed inoculation with bacteria cause a significant increase in the average number of pods in plant by 11 percent compared to the non-inoculated with bacteria. As can be seen in Table 2, the effect of cultivar in the genotype of the number of pods in plant was highly significant ($P < 0.01$), So that Among the studied cultivars, M7 was the maximum (59.17) and Sahar was the minimum(45.5) number of pod in plant, that showed the increase of 23.1 percent of the number of pods. This is probability associate with the premature, Classification of several branch and desirable genetic characteristics for cultivars M7, in terms of better compatibility with symbiotic bacteria and optimal use of environmental conditions associated. In investigating of studied cultivars of characteristics of numbers of pods in plant in the level of 0 percent, there is significant differences.

They announced that among the five cultivars, the highest number of pods in plant is observed in limited growth 0.32 and L14. This is due to the differences of cultivars of plant in terms of tributaries. This means that the average number of produced branches in limited numbers and thus the number of produced pods in tributaries in this cultivars considerably is more than the number of indeterminate growth, Williams and DPX. In research (Gan *et al.*,2006), on the effects of cultivars inoculation of forage soybean with different strains of Brady bacteria *Rhizobium japonicum* reported that inoculated cultivars, could create more performance and more pods number. In other experiments that is done on bean cultivars inoculated with different strains of bacteria, it is mentioned that there is a significant difference in the number of pods in plant. That among the compounds of cultivar and bacteria, combination of beans of Maurada cultivar and strains of bacteria Semia 481 created the highest rate of pods in plant. Also in experiment of Akbari *et al* (2010),in research of seed inoculation of soybean cultivars with different strains of bacteria, the obtained results showed that, Soybean cultivars have significance difference in terms of number of pods in plant.

C. Number of seeds in pod

Obtained results of analysis of variance in this testing reveals significant differences of number of seeds in soybean pods affected with inoculated treatment with Rhizobium bacteria is in the probability of 1 percent. As inferred from the table is the average comparisons, inoculated treatment increased 23% the number of seeds in pod, compared to non-inoculated with bacteria. obtained results in relation to the number of seeds, in pod in affected of inoculation is in correspond to the results of Kazemi, Comparison of means in inoculated condition with bacteria and non-inoculated with bacteria in their study showed that seeds inoculated with bacteria cause a significant increase in the mean of seeds in plant by 14 percent compared to the non-inoculated with bacteria. Also, the effect of cultivars on the number of seeds in pod was significant at 0% level. So that, among studied cultivars, the highest number of seeds in pod was obtained in (4.00) from Sahar cultivars that Statistically, is located with Zan cultivars in a class, number of seeds in pod is main component in determining the performance of soybean seed. This factor was in the control of plant genotype and less is affected with environmental factors. In investigating studied of Asa *et al.*,2006 showed significant difference in Number of seeds in pod at 0% level. They declared among the studied cultivars, Williams had the highest number of grains in pod. They stated in their review, genotype of Seeds in pod did not show significant differences that was inconsistent with this results, While there was significant difference between strains of bacteria were placed in different groups. So that the combination of lines 11 and acetic bacteria with 3/9 seeds in pod was superior to the others that shows the desired effect of the bacterial strains.

D. Biological performance

The results showed that inoculated effect of soybean seed inoculation with bacteria with bacteria *Rhizobium japonicum* on biological performance was significant at 0% level, , So that maximum biological performance was obtained in inoculated treatment of seed with an average of 2 kg per hectare, respectively. In fact, the inoculation cause increase of vegetative development, plant height, number of branches, number of leaves and leaf area and especially the biological performance of soybean. Cultivars effect on biological function was significant. So that among the tested cultivars, most biological function of cultivars M7, with mean of 4290/5 kg per hectare and minimum cultivars of Zan with mean2509/8 was obtained. That put in one group with Zan cultivar statistically. The results were consistent with the results of Hadi *et al.*, 2002. In their research, obtained plant of the seeds of cultivar Menukin inoculated with soybean inoculums had more dry weight. (Mendham and Scott, 2003) believe that the most effective soybeans cultivars to coexist with bacteria are groups that transform Higher percentage of generated Asymylat for the use of nitrogen-fixing bacteria to root, Also mentioned that the use of bio fertilizers and inoculums can increase biological functions in Legume. They showed Capacity of stimulating plant growth and product dry weight through direct mechanisms produced by different soybean of rhizobium. Asadi Rahmani *et al.*2004 also on a review on soybeans showed that inoculation significantly increases dry weight also they founded on their studies on soybean that inoculation of different races of *Rhizobium japonicum*, increased dry matter production significantly. Reported that in the seeds inoculated with growth bacteria, biological function increased compared to non-inoculated control. And has an increase of eight percent in this case rather than the lack of inoculation.

E. Seed function

Summary Analysis of variance showed that inoculation on grain function at 1% level of the soybean cultivars was significant. The highest performance was related to the inoculated treatment with function of 112 with 6% increase compared to the inoculation treatment also affect the performance of soybean cultivars increase them.

So that the maximum performance of the seed was obtained from M7 cultivars 1461 kg hectare / a Average 0 / minimum of the Sahar cultivars was obtained with function of 61 kg per hectare, respectively. It seems that the increase of performance in pretreatment of seed. With bacteria due to increase of availability of food intake, increase of root health during the growth period in competition with pathogens root. beneficial effects of inoculation with bacteria on the performance of cereals, legumes, oilseeds, fruits and vegetables have been reported by many researchers. In the study of Kazemi *et al,*2008 in relation to the impact of seed inoculation with bacteria on performance and components of soybean performance, reported that inoculation significantly increase the final performance of soybean. The rate of increase of seed performance in inoculation conditions on average compared to control (Non-inoculated)was 25 per cent. Also there was a significant difference between cultivars in terms of grain performance, in expressing the reason of this, they noted to, being late growth high potential of 25 lines than Williams cultivars. (Hungria and Bohrer, 2000) in their study on the performance of soybean cultivars inoculated with different strains of with bacteria *Rhizobium japonicum* reported, significant differences among soybean cultivars. In the culture of Asa*et al,*2006 grain performance of tested cultivars at 1% level showed a significant difference. They announced that among the studied cultivars, limited growth cultivars had the most performance. And knew the reason of it limited and unlimited growth type of cultivars. So that limited growth of cultivars have more performance due to forming more pods than cultivars of unlimited.

F. Harvest index

The results of analyzing variance showed that the was not affected by inoculation. But this trait at 1% probability level was affected by cultivars. So that the most harvest indicator of cultivar M7, L17 with mean 33,70 and 34/19 and the minimum of them with the 30.68 of Williams cultivars was. That was consistent with the results of Yadgari *et al* 2009. in the found that similar experiments that has done by Asadi*et al* on the soybean and Strains of bacteria *Rhizobium japonicum* also pointed significant differences in dry weight of soybean cultivars. Rodriguez *et al.,* 2007 reported that there is no significant difference in dry weight values of Kanilny bean cultivars in inoculation with different strains of Rhizobium and also Vasilas, 2007, on soybean (Hafeez *et al.,* 2000), on lentils, and test results) Carranca*et al.,*2009 (on peas, peas and beans),Antoun et al., 2010, on radish shows significant difference on the dry weight of inoculated cultivars than un inoculated. Mahdipour *et al* 2008 examined the effect of symbiotic bacteria in shoots and seed function in soybean and concluded that Inoculation of bacteria increases the growth and nodulation in soybean. Meghvansi *et al.,* 2005, reported that inoculation with *Rhizobium* strains can improve the vegetative growth in soybean. Plants obtained from the optimum irrigation of cultivars Menavkin inoculated with inoculums of soybean has higher plant dry weight. And compared to non-inoculated increased 51 percent.

Table 2: Summary of the variance analysis results of performance and components of soybean cultivars influenced by inoculation with bacteria *Rhizobium japonicum.*

Measured characteristics							Degrees of freedom	Sources of variation
Harvest index	**Grain function**	**Biological performance**	**Seed weight**	**Seeds per pod**	**Pods per plant**	**Plant height**		
0.37ns	20921.36*	15.280011ns	2.65ns	0.11**	147.86ns	17.88ns	2	Repeat
0.95ns	156420.25**	1655082.25*	0.55ns	2.25**	11.336ns	50.65ns	1	Insemination
3.18	509.25	28322.33	1.94	0.001	19.32	5.95	2	The first error
9.71**	465792.23**	3353862.09**	0.40ns	0.83*	11**.140	70.59**	5	Variation
5.05ns	2448.45ns	48841.52ns	0.39ns	0.12ns	18.11ns	15.34 ns	5	Variety *Insemination
0.75	5080.81	47644.16	0.27	0.29	8.63	17.12	20	The second error
2.63	6.34	6.41	6.42	5.24	5.75	7.77	-	The coefficient of variation

* and ** and ns is significant at 1% , 5% level

Also Zahir *et al.,* 2004, reported that increasing hormone of growth secreted by increasing bacteria of plant growth by stimulating the growth of bacteria increases the seedling dry weight and its components. The current results show that the most studied characteristics are in affection of inoculation and cultivars. Generally, we can conclude that inoculation on have important role on performance and components of seed and can increase that. As a result, with the goal of planting soybeans (an increase of quantity or quality) can choose the best cultivar or the best inoculated combination of cultivars. As the results showed, inoculated cultivars M7 had the most biological performance and produced the seed performance.

Table 3: The comparison of performance characteristics components of soybean cultivars affected by inoculation with bacteria this *Rhizobium japonicum.*

Measured traits				
Grain performance (Kg per hectare)	**Biological function (Grams per Plant)**	**Seeds per pod (Number)**	**Pods per plant (Number)**	**Treatment**
1058.61b	3190.94b	3.28b	48.00b	Non inoculated
1190.44a	3619.78a	3.78a	54.11a	inoculated

The mean of each column with the same letter are not significantly different.

Table 4: Comparison of the means of performance Characteristics, components of soybean cultivars Affected by soybean cultivars.

Measured characteristics						Treatment
Harvest index (Percentage)	**Grain yield (Kg per hectare)**	**Biological function (Grams per Platt)**	**Seeds per pod (Number)**	**Pods per Plant (Number)**	**Plant height(centimeter)**	
34.19a	1467.50a	4290.5	3.33ab	59.17a	50.03b	M7
32.73b	820.67d	2509.8d	4.00a	45.50d	53.98ab	Sahar
30.68c	951.67c	3115.2c	3.67ab	50.67bc	49.98b	Williams
33.36ab	1330.50b	3987.3b	3.00b	53.83b	51.85b	M9
32.56b	861.17d	2653.3d	3.83a	48.67cd	59.08ab	Zan
33.97a	1315.67b	3875.7b	3.33ab	48.50cd	54.38ab	L17

The mean of each column with the same letter are not significantly different.

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