9

Symbiotic Nitrogen Fixation in Soybean

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1. Introduction

Nitrogen is the key component of vegetable protein for human and animal consumption. Although 78% of the atmosphere by volume is consisted of molecular nitrogen, this huge amount is not available for plants, animals or human. Only the bacteria that have nitrogenase enzyme can reduce atmospheric nitrogen and thus they called as a "nitrogen fixers". Nitrogen fixers reduce molecular nitrogen to amino acids and protein through ammonia (Fritsche, 1990; Lindemann and Glower, 2003). Nitrogen fixation process realizes either by free-living, associative or symbiotic nitrogen fixers. In symbiotic relation microorganism infects the plant root through infection thread and lives in the nodule forming structure. Afterwards plant supply component of nitrogenase and organic compounds to microorganism whereas microorganism supply reduced nitrogen to plant. Associative microorganisms are not infecting to plant however they colonize in rhizosphere and use of root exudates to successful nitrogen fixation. Free living fixers are independent and they need neither infect to plant nor rhizosphere exudates for fixation. Although the fixation rate vary depends on the nitrogen fixer type, the most effective fixation occurs in symbiotic relation with legumes. Soybean itself represents 77% of the N fixed by the crop legumes by fixing 16.4 Tg N annually, fixation by soybean in the U.S., Brazil and Argentina is calculated at 5.7, 4.6 and 3.4 Tg, respectively (Herridge et al. 2008).

Plant and microorganism are particular for each other, thus only certain microorganism can infect particular plant whereas the appropriate rhizobium of soybean is called as *Bradyrhizobium japonicum*. The shape and size of the nodules are also particular for legumes, the soybean nodules are round and in same cases big as pea. Effective nodules are large in size and reddish in the inside colour.

Legumes have an important role for both human nutrition and animal feeding, however, soybeans are unique in legumes with contents of 40% protein and 21% oil as well as isoflavones. Thus, soybean is the most widely grown protein/oilseed crop in the world, with both North and South America producing large portions of the world's supply of this remarkable crop.

In case of legume introduce to soil for the first time, appropriate rhizobium strain has to be inoculated for successful nitrogen fixation. In many cases some rhizobium bacterium might be existed in the soils, nevertheless, due to the insufficient number and activity (Gok and Onac, 1995), inoculation should be repeated. No successful nodulation as well as nitrogen fixation should be expected without inoculation with appropriate and healthy rhizobium strain by convenient inoculation method. A number of methods available to used in inoculation of soybean by *Bradyrhizobium japonicum*, however, inoculation by irrigation water and seed bad inoculation methods are more effective according to nitrogen fixation parameters (Isler and Coskan, 2009). On the other hand organic compound such as fulvic and humic acids have stimulatory effect on soybean-rhizobium symbiosis (Coskan et al., 2010). Moreover, biological nitrogen fixation of soybean influenced by the number of factor such as pH, salinity, partial oxygen pressure, soil water content, ambient temperature as well as soil mineral N content.

2. Cultivation of soybean for a first time: In scope of inoculation view

Cultural plants need considerable amount of macro and micro nutrients in mineral form to produce high quality of yield and these nutrients should be provided to correct yield-limiting factors. Mineral and organic fertilizations are the pathways to enhance soil mineral nutrient budget. Due to the plant can only use mineral forms of nutrients, mineral fertilizers are readily available for the plants. Nutrient in organic fertilizers are in organic form that not readily available for the plants, thus the organic fertilizers have to be convert mineral form via the process called “mineralization”.

Considerable amount of nitrogen is removed from soil when protein-rich grain or hay harvested, thus nitrogen is the most commonly deficient nutrient among macro and micro nutrients. Due to the nitrogen is the key component of healthy growing, all plants other than legumes should be fertilized by nitrogenous fertilizer. Legume plants are unique for their ability to fix nitrogen from atmosphere by symbiotic relationship with rhizobium bacteria. Rhizobia require a plant host therefore they cannot independently fix nitrogen. These bacteria located around root hair and fixing atmospheric nitrogen using particular enzyme called “nitrogenase”. When this mutualistic symbiosis established, rhizobia use plant resources for their own reproduction whereas fix atmospheric nitrogen to meet nitrogen requirement of both itself and the host plants. Supply of nitrogen through biological nitrogen fixation has ecological and economical benefits. Farmers are not taking advantage of rhizobial inoculation to a number of reasons, thus they are passed up the potential of biological nitrogen fixation.

In many cases *Rhizobium spp.* might be existed in the soils, nevertheless, due to the insufficient number and activity (Gok and Onac, 1995), inoculation should be repeated. A number of studies indicate that no nodule formation appeared in the soybean roots if inoculated soybean isn’t grown previously. Biren (2002) carried out the experiment to evaluate the effects of rhizobium inoculation in Turkish Republic of Northern Cyprus where soybean is not cultivated previously. He reported that there was no nodule formation in the non-inoculated control plants. Similarly, in Isparta where the soybean is not cultivated regularly there was no nodule occurrence (Coskan et al., 2009). In some circumstances it is possible to observe very limited number of infection even in first cultivation at non inoculated condition. Isler and Coskan (2009) reported that in the first cultivation in non-inoculated condition there was a very few nodule formation in very light weight.

In scope of inoculation view, rhizobium inoculation should be realized with appropriate and healthy rhizobium strain in first cultivation of soybean plant and inoculation should be repeated every 2 to 3 year to sustain successful symbiotic nitrogen fixation. Depends on the rhizobium variety used, amount of nitrogen fixation greatly changed (Gok et al., 2001; Coskan et al., 2003). Thus, results obtained from local research should be considered in designating the effective strain.

3. Effects of inoculation methods on fixation

A number of inoculation methods are available, however, wetting the seeds by sugar, water and strain mixture or inoculation with peat culture are the most common methods in practise. Due to the rhizobium strain sensitive to the sunshine, inoculation and drying should be realized in indoor environment and seeds should also be protected from direct sunlight at sowing. Inoculation by wetting the seed methods has a number of disadvantages as follows: (1) Seeds are clinging to each other or to any surface of sowing equipment. Thus, farmers are abstained from inoculation to prevent time loss and extra workload. (2) The use of excessive water damages to the shell of the seed during inoculation therefore seeds become vulnerable to external conditions. Deaker et al. (2004) reported that seed inoculation method causes reduction of viable cell number when seed passes through machinery or lifting the seed coat out of the ground during germination. (3) Less amount of strain can be introduced to the seed especially in smaller seeds. Therefore, the higher amount of rhizobium bacteria per seed can be used in soil inoculation method compared to seed inoculation, especially for small seeded legumes (Brockwell, 1977).

Isler and Coskan (2009) tested the five different inoculation practises in pot experiment to evaluate the most effective method. They use the methods as follows: seed inoculation with sugar as an adhesive (SI), top inoculation with first irrigation (TI), two times top inoculation, one with first irrigation and one after germination (TTI), seed bad inoculation (SBI) and inoculation with peat culture-rhizobium suspension IWP). Result revealed that all practices other than control increased both number of nodule and nodule weight (Fig. 1). SI which commonly used inoculation technique was not effective as the other techniques tested. Observed nodule formation in TI proved that inoculants may reach rhizosphere area without any difficulties. Therefore inoculation with irrigation water may be used as an alternative inoculation technique considering the salt contents of the irrigation water. TTI application realized to compare with TI, however there wasn't statistical differences between TI and TTI.According to yield and the weight of seeds, SBI was the most effective inoculation techniques. Moreover SBI is the method that can easily adapt to sowing machinery with small changes.

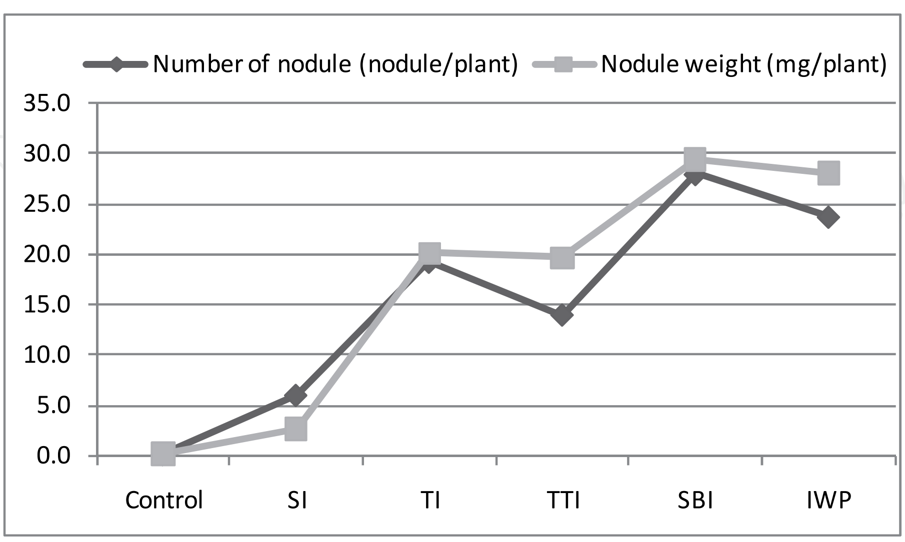

Fig. 1. Effects of inoculation methods on nodule formation and nodule weight (SI: seed inoculation, TI: top inoculation, TTI: two times top inoculation, SBI: seed bad inoculation, IWP: inoculation with peat culture)

In this method seed is not directly contacted to the inoculants material, instead, seed located nearby or above the soil which rhizobia applied. Thus, the difficulties reported by Deaker et al. (2004) and Brockwell (1997) surmounted. Due to the inoculation material mostly stored in peat culture, inoculation with peat culture is another common inoculation method. But, if the peat culture dries out after inoculation, peat removed from seed and accumulate bottom of sowing machine (Gault 1978). Besides, when dry peat is wetted, great heat occurred which may reduce the number of viable rhizobia (Deaker 2004). Thus, in IWP application, water added to peat before adding the rhizobia to prevent high temperature occurrence, then this suspension applied to seed bed. Nodule count and nodule weight results revealed that the problem mentioned above is not realized and effective infection occurred in IWP. Results strongly indicate that, in the case of inoculants were not contact with seeds directly, the success of symbiotic relation increased. In general, SBI were the most effective methods among all methods tested. This method is also ripe for development of automated sowing machines.

4. Effects of different tillage system

Biological N_2 fixation (BNF) was effected by different tillage system including agricultural practices, pesticides applications, addition of organic material, residue chopping. The ways in which these operations are implemented affect the physical and chemical properties of the soil, which in turn affect soil microorganisms as BNF bacteria.

The amount of nitrogen actually fixed by a legume depends not only on the genetics of the bacteria and host plant but also on the environment and agricultural practices. Among the common agricultural practices, fertilization with P and N has important effects in nitrogen fixation. It is a well-established fact that, when legumes are grown in soils high in available nitrogen, the nitrogen fixation rate is reduced.

According to different research, by definition, biological N_2 fixation (BNF) is synonymous with sustainability. Advances in agricultural sustainability will require an increase in the utilization of BNF as a major source of nitrogen for plants. The process of BNF offers an economically attractive and ecologically sound means of reducing external nitrogen input and improving the quality and quantity of internal resources.

Soil tillage methods have complex effects on physical, chemical and biological properties of soil. Because of the changing physical and chemical properties of soil by soil tillage methods, the biological properties of soil may also change. Actually these changes are indirect results of tillage. Changed physical and chemical soil properties by soil tillage methods effect the parameters directly related with soil microbial activities such as organic matter, soil humidity, temperature and ventilation as well as the degrees of interaction between soil mineral and organic matter. As a result of these effects, significant differences can be observed in the population of microbial activities in soil (Kladivko, 2001; Lavelle, 2000; Wardle 1995; Saggar et al. 2001).

Plant and microorganism interactions in rhizosfer region are very important for plant growth. In the rhizosphere region, rhizobial activities occur as reciprocal and compulsory interactions (symbiosis) of plant-microorganism (Altieri, 2000; Garcia and Altieri, 2005). One of the important activities related to soil qualities is beneficial microorganism activities. The most important of these activities is a root nodule bacterium which provides to biological N_2-fixation (Ferreira et al., 2000).

Microorganisms, that are important parts of the nature, are considerably affected by the environmental conditions. These organisms which rapidly reproduce and function in proper

environmental conditions, also struggle to continue their functions under poor conditions (Doğan et al., 2007).
As a result of symbiotic N_2–fixation, legumes supply nitrogen to the soil not only with their nodules, but also by decomposition of their roots and shoots. Nitrogen might have formed by mixing the separated dead nodule tissues into the soil. This situation can be accelerated by cutting of the plant's shoots (Werner, 1987; Goormachting et al., 2004).
In a study of Dogan et al. (2011), the effects of six different soil tillage methods (Table 1) on some parameters related with nitrogen fixation have been investigated. According to the findings of the research in the No-Tillage with Direct Seeding (NTDS) plots, root weights (6.9 g/plant), number of nodules (96 number/plant), weight of nodules (0.318 g/plant) and root nitrogen content (% 0.71) are found to be statistically higher than with the other tillage applications. In the Reduced tillage with rotary tiller (RTR) plots, the values of up-root dry weight (51.3 g/plant), mean nodule weight (3.91 mg/nodule), root N content (2.38%), are found higher on the lands than in NTDS plots.

Soil Tillage Methods	Soil Tillage for winter wheat cultivation	Soi Tillage for second crop soybean plant
Conventional Tillage with Residue (CTR)	Chopping the residues Plowing (30-33 cm) Disk horrow (13-15 cm) (2 times) Packing (2 times) Wheat planting with a universal planter (4 cm)	Chopping the residues Heavy disk horrow (18-20 cm) Disk horrow (2 times) (13-15 cm) Packing (2 times) Soybean planting with Pneumatic-precision seeding machine (8 cm)
Conventional Tillage with Burnt Residue (CTBR)	Burning the residues Plowing (30-33 cm) Disk horrow (13-15 cm) (2 times) Packing (2 times) Wheat planting with a universal planter (4 cm)	Burning the residues Chiselling (35-38 cm) Disk horrow (13-15 cm) (2times) Packing (2 times) Soybean planting with Pneumatic-precision seeding machine (8 cm)
Reduced Tillage with Heavy Disking (RTHD)	Chopping the residues Heavy disking (18-20 cm) (2 times) Packing (2 times) Wheat planting with a universal planter (4 cm)	Chopping the residues Rotary tilling (13-15 cm) Packing (2 times) Pneumatic-precision seeding machine (8 cm)
Reduced tillage with rotary tiller (RTR)	Chopping the residues Rotary tilling (13-15 cm) Packing (2 times) Wheat planting with a universal planter (4 cm)	Chopping the residues Rotary tilling (13-15 cm) Packing (2 times) Soybean planting with Pneumatic-precision seeding machine (8 cm)
No-Tillage with Heavy Disking (NTHD)	Chopping the residues Heavy disking (18-20 cm) Doting (2 times) Wheat planting with a universal planter (4 cm)	Chopping the residues Herbicide application Soybean planting with Pneumatic-precision seeding machine (8 cm)
No-Tillage with Direct Seeding (NTDS)	Chopping the residues Herbicide application Wheat seeding with direct seeder (4 cm)	Chopping the residues Herbicide application Soybean planting with Pneumatic-precision seeding machine (8 cm)

Table 1. Soil tillage methods in the major and secondary crop (soybean) production (Dogan et al., 2011)

Among the applications, in the plots of Reduced Tillage (RTHD and RTR) rhizobial nitrogen fixation parameters have been found considerably higher compared with the other applications (Fig. 2). However, some soil tillage methods used in this study negatively affected some soil parameters. For the Reduced Tillage with Rotary tiller (RTR) plots the dry root weight (4,8 g/plant), up-root weight (35,7 g/ plant) and root N content (% 0,68) values and for the Conventional Tillage with Burnt Residue (CTBR) plots, number of nodules and weight of nodule values were found to be lower than in the other tillage applications. The values of dry nodule weights, like in Conventional Tillage with Burnt Residue (CTBR) were low in the plots of Conventional Tillage with Residue (CTR) and Reduced Tillage with Heavy Disking (RTHD) with the values 0,071 and 0,088 g/plant, respectively. Besides, the lowest mean nodule weights (2,06 mg/nodule) have been observed in Conventional Tillage with Residue (CTR) plots and the lowest up root N content (%1,98) have been observed in Reduced Tillage with Heavy Disking (RTHD) plots.

The results of the study have been showed that, parameters of nitrogen rhizobial fixation has been affected negatively by the conventional tillage methods in which 3-5 tillage operations are applied and soil is disturbed . There were differences among the tillage methods and these differences were found to be statistically significant. In general, the best results related with rhizobial activity have been obtained with No-Tillage with Direct Seeding (NTDS) and No-Tillage with Heavy Disking (NTHD). However, other soil tillage methods decreased the nitrogen fixation (Dogan et al., 2011). Similar studies have also showed that zero and reduced soil tillage methods have increased the soil microbial activity and population (Ferreria, 2000; Alvarez et al., 1995; Gassen and Gassen 1996).

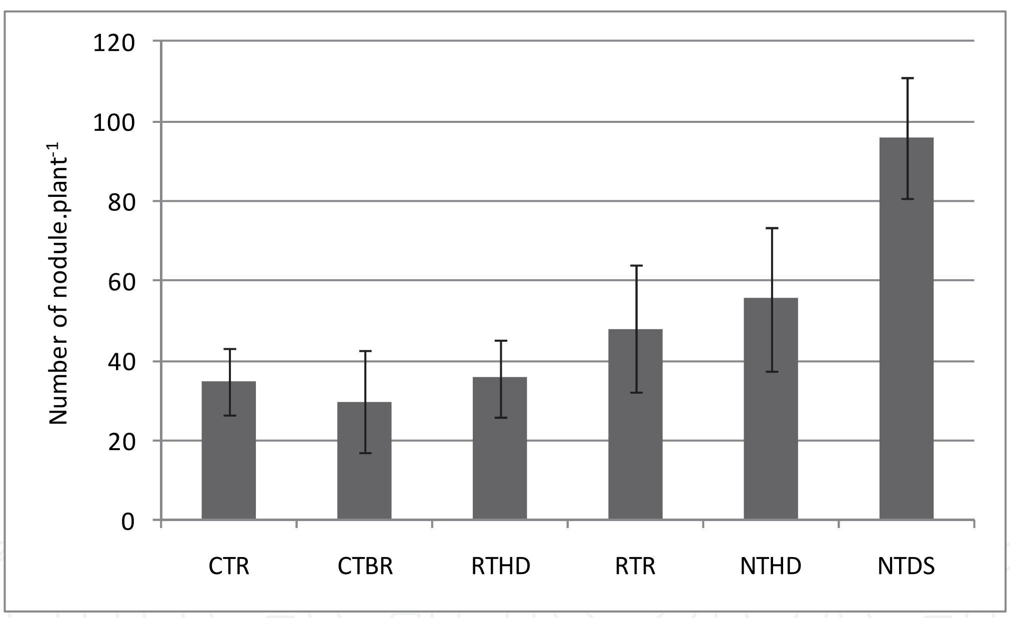

Fig. 2. The effects of different soil tillage methods on number of nodule in secondary crop soybean plant (CTR: Conventional Tillage with Residue, CTBR: Conventional Tillage with Burnt Residue, RTHD: Reduced Tillage with Heavy Disking, RTR: Reduced tillage with rotary tiller, NTHD: No-Tillage with Heavy Disking, NTDS No-Tillage with Direct Seeding)

Generally, soil microbial activity is affected negatively by soil tillage (Jinbo et al., 2007; Kladivko, 2001; Hussain et al., 1999; Saggar et al., 2001). Therefore rhizobial activity is also be affected negatively by soil tillage (Hassen et al., 2007; Ferriera et al., 2000). Soil organic matter decreased by soil tillage operations is also important for the vital activities of soil microorganisms. The decrease of organic matter in the soil can also cause decreases in soil microbial activity (Saggar et al., 2001; Eliot el al., 1984). As it can be seen in the similar studies, the effects of soil tillage methods may differ depending on climate, regional, and environmental factors. These factors must be taken into consideration before applying tillage methods. Otherwise, biological activity, fertility and sustainability of soil will be destroyed.

On high-input farms, microorganisms are generally thought to play a minor role in soil fertility because most nutrients in inorganic fertilizers are readily available for the plants and do not require degradation or mineralization (Smith et al., 2001).

Many studies have concluded that herbicides affect nitrogen fixation largely via indirect effects on plant growth and consequent availability of photosynthate to the root nodules (Wally et al., 2006; Abd-Alla et al. 2000); there is evidence that some pesticides might impair the ability of the rhizobia to recognize appropriate host plants. As a consequence, early nodulation events can be disrupted. However, according to their research, not all pesticides had a negative impact on nodulation and the degree to which nodulation was inhibited was dependant on pesticide concentrations. In some instances, results from various studies have been contradictory. For example, when examining the effects of chlorsulfuron under laboratory conditions, Anderson et al. (2004) observed that even at rates equivalent to two times field rates, chlorsulfuron did not influence rhizobial growth. However, although rhizobial growth was not influenced, the subsequent ability of these rhizobia to form nodules was reduced. Thus, they reported that when rhizobia were exposed to relatively high levels of chlorsulfuron, subsequent nodule size and total nitrogen fixation was reduced. In contrast, Martensson (1992) reported that nodulation ability was unaffected by previous exposure to chlorsulfuron. These contrasting results suggest that the impact of various herbicides on specific nodulation events may be highly dependent on specific environmental conditions, including different soil characteristics (i.e., pH, organic matter, moisture, etc.) and weather conditions. Martensson (1992) examined the impact of various herbicides on root hair formation. Rhizobia infect plant roots through root hairs and thus it was hypothesized that herbicides affecting root hair development might interfere with nodulation. Author reported that some herbicides, including glyphosate, caused root hair deformations that apparently resulted in fewer nodules being formed. It is important to note, however, that this was a laboratory study and consequently the herbicide rates used in these experiments were not necessarily similar to rates that would be encountered in soils under field conditions. Thus, although the research demonstrates the possibility for herbicides to affect nodulation via root hair deformations, it is not known if this phenomenon occurs under field conditions (Walley et al. 2006).

Saggar et al. (2001) studied the effect of cultivation on soil organic C, functional chemical composition of SOM, and soil structure in soils of contrasting mineralogy. They found that soil susceptibility to structural degradation increased with years of cultivation, and from light textured to heavier textured soils. Because cultivation causes profound changes in the soil physical and chemical properties, and populations of microfauna and macrofauna, it is relevant to quantify its effects on soil microbial and microfaunal populations and on SOM dynamics.

5. Mycorhiza-rhizobium interaction in light limited condition

Vesicular Arbuscular Mycorrhiza (VAM) is symbiotically living organism with many crops and they enhance plant P uptake along with other micronutrients especially Zinc. Phosphorus efficiency in highly limy soil (in high pH) is considerably low whereas mycorrhiza assists plant to receive that immobile phosphorus by exudates and/or enhancing soil contact area. As mentioned previously, rhizobium is a microorganism, capable of fixing aerial nitrogen (N_2) to soil/plant via symbiotic relations with legumes. Both organisms utilize the photosynthesis products that assimilated by host plants to survive. In non-limiting conditions those organism supports the plants for the most important macro and micro nutrients as N, P, Fe and Zn. On the other hand unsuitable soil or climatic conditions in growth season may result negative Rhizobium x Mycorrhiza interactions. Although both microorganisms use organic compounds formed by plants, they use trace amount of that compound compared to the plant biomass formation. Coskan et al. (2003) carried out the pot experiment to evaluate cross interactions of rhizobium and mycorrhiza in light limited condition. Results revealed that no nodule formation appeared in non-rhizobia-inoculated control variant whereas rhizobial inoculation increased number of nodule (Fig. 3) while decreased biomass weight (Fig. 4).

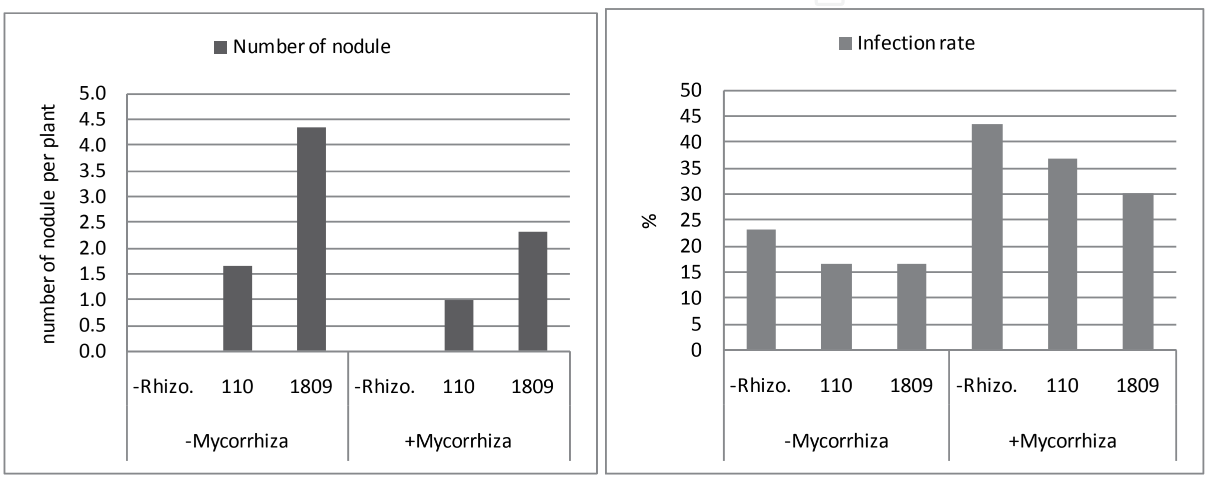

Fig. 3. Nodule formation (left) and mycorrhizal infection rate (right) in the light limited condition

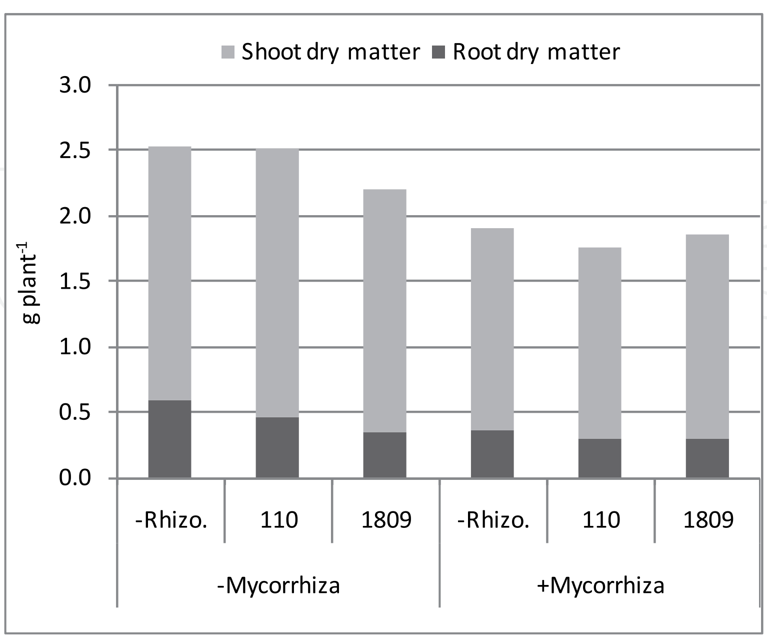

Fig. 4. Effect of dual inoculation on root and shoot dry weight of soybean in light limited condition

Although both of the rhizobium strains give rise to nodule formation, *B. japonicum* 1809 strain cause considerably higher nodule number. On the other hand, mycorrhiza inoculation increased the infection rate (Fig 3). Rhizobial inoculation decreased mycorrhizal infection rate in both with mycorrhiza and without mycorrhiza applications. Bacterial inoculation has no significant effect on plant growth except nodule formation. It is clearly seen that both rhizpbium and mycorrhiza applications reduced total plant dry weight. However, plant dry weight and phonological observations revealed that plant development is adversely effected due to mycorrhizal inoculation in light-limited growing session.

6. Effects of humic+fulvic acid on symbiotic nitrogen fixation

Organic matter is one of the most important issues of agriculture and it contains three very important components: humic acids, fulvic acids and humin. Plants and microorganisms in soil benefit from applications of humic acid in several ways. Humic acid stimulate root growth, increase carbohydrate production, have a hormone-like affect within the plant, and increase soil microorganisms (Lawn Care Academy, 2010). The incorporation of humic acid fractions in media designed for the enumeration of soil micro-organisms belonging to specific physiological groups was found to result for some groups in appreciably higher counts. It is suggested that by influencing the enzyme systems of certain micro-organisms, humic compounds may affect the range of substrates which they can utilize. The effect could have implications on the activity of organisms in environments in which humic substances are normally present, such as soils and natural waters (Visser, 1984).
Coskan et al. (2010) carried out a pot experiment to represent effects of humic + fulvic acid (HFA) applications on biological nitrogen fixation under soybean vegetation. Humic + fulvic acid application realized by either incorporate to soil or admixing by irrigation water. Seeds are inoculated by appropriate Bradyrhizobium japonicum strain, before sowing. In flowering stage, roots are removed from soil and the number of nodule determined (Fig 5).

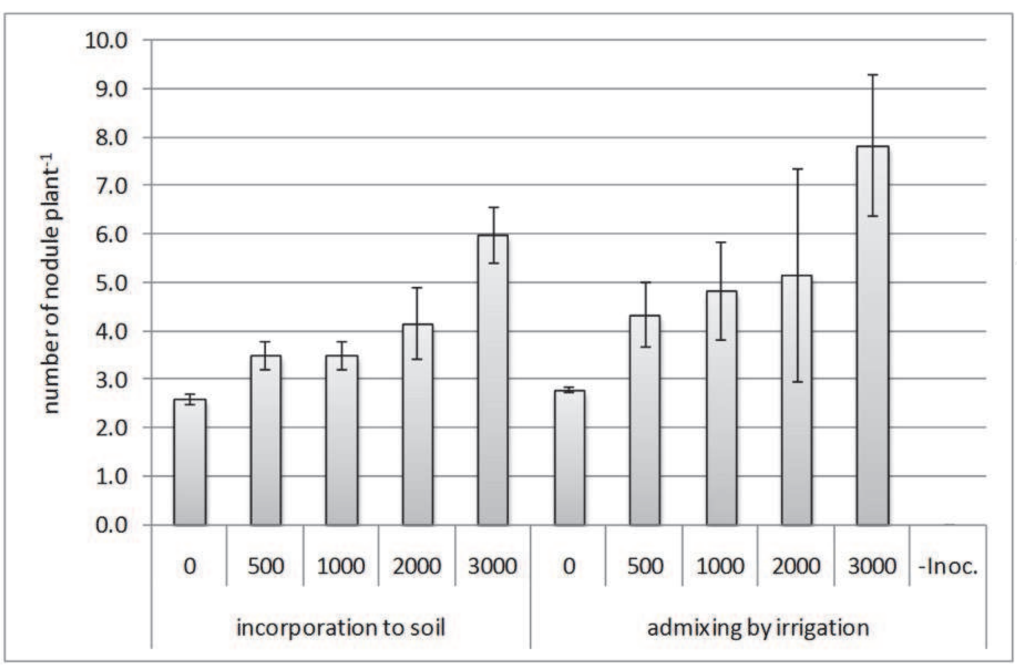

Fig. 5. Effects of humic + fulvic acid application on nodule number of soybean (-Inoc: non-inoculated)

Due to the fields where soil was taken is not previously introduced with the rhizobia that match with soybean; no nodule occurrence was observed in the pot which non-inoculated control variant. A few nodules observed in non-HFA applied pots however HFA application increased nodule occurrence considerably. Both "incorporation to soil" and "admixing by irrigation" applications were effective on formed nodule number; however because of the dilution effect in incorporation to soil application, admixing by irrigation application seems to be more effective than incorporation to soil. (dilution effect). Increasing doses of HFA increased the number of nodule, thus findings expressing the considerable positive effects of HFA on biological nitrogen fixation.

7. Effects of organic matter application

Soil organic matter is important for biological nitrogen fixation (BNF) because of its influence on soil physical, chemical and biological properties and processes. It helps to create a favourable medium for physical processes, chemical reactions, and biological activity. The multi-faceted role of soil organic matter (SOM) must therefore be taken into consideration in any assessment of `soil quality' and `sustainable land management. Low concentration of organic matter can have a deleterious effect on offsite environment because it is often associated with decreased soil fertility, water holding capacity and water infiltration, and increased erosion. Further, SOM turnover controls the fluxes of nutrients. Microbial biomass measurements combined with soil respiration have frequently been used as an index of soil development or degradation (Insam and Domsch, 1988) and to assess the quality of organic matter input (Anderson and Domsch, 1990). Interactions between soil microorganisms and soil microfauna, particularly Protozoa and Nematoda, largely control SOM turnover (Elliot et al., 1984).

Harvested crop residues and rotation has a major impact on the soil organic matter content. However, some features, such as the type and degree of decomposition of organic matter, affected BNF in different ways. Many field trials which were applied organic material by Gok et al. (2001) show that with the organic material application, organic matter content of soil increased in the short term but at the end of the trial, soil organic matter content decreased due to high rate of mineralization. To gain long-term soil organic matter, all kind of organic substrates should be regularly added to the soils which under the effect of semi-arid climate condition. Moreover, degradation resistant substrates such as lignin and cellulose should be preferred to dump mineralization. With this way nitrogen is temporarily immobilized in biomass that preventing (Asmus and Hubner 1985; Gok 1987). In a research, Limon-Ortega et al. (2002) studied to evaluate the effects of burning and natural wheat or maize stubble on some properties of soil. Results indicated that the positive effect of that substrates appeared after 2 or 3 year continuously stubble applications. The result obtained at 5th – 6th years were more expressive than those obtained in the 1st to 3rd years. When the stubble is burned almost all nutrients in organic substrates converted to available form for plants in seconds. Therefore, compared with burned or natural stubble applied plots, in the beginning years burned stubble seems to be more efficient, but in following years the effects of stubble become much more effective on the soil parameters.

A two year field experiment at soybean cultivation was undertaken for determining the effects of stubble burning, a widely performed practice in Cukurova Region, along with admixing 0, 5000 and 10000 kg ha^{-1} tobacco wastes on symbiotic nitrogen fixation, grain yield and biomass production (Coskan et all, 2009). Results revealed that applications were significantly effective on nitrogen fixation, yield and biomass production. According to overall averages, the highest biomass production of root and shoot were observed at wheat burned and 10000 kg ha^{-1} tobacco waste applied plot as 830 and 4730 kg ha^{-1}. The highest nitrogen contents at harvest stage were determined in the plot wheat and 5000 kg ha^{-1} tobacco waste applied (root, 0.87%; shoot, 0.95%). At the end of experiment determined grain yield amounts in first year were higher in the stubble burned plots. No statistical difference was determined between burned and non-burned stubble in the second year. When the variants of tobacco waste applications were compared according to their tobacco rates, the productivity was increased at plots of waste application in both years. The determined highest yield 4520 and 5280 kg ha^{-1} at stubble burned and non-burned plots in which 10000 kg ha^{-1} waste was applied in the first and second years, respectively

8. Factors that effective on symbiotic nitrogen fixation in soybean

Nitrogen fixation is one of the important soil microbial activity which was affected by all ongoing processes in soil as well as other soil microorganisms. The biological nitrogen fixing process depends on the occurrence and survival of Rhizobium in soils and also on their efficiency (Adamovich, Klasens, 2001).

The rate of the nitrogen fixation was affected by many different physiological and environmental factors in soil, such as temperature, water holding capacity, water stress, salinity, nitrogen level, pH and other nutrients. Many of these factors, including temperature, affect many aspects of nitrogen fixation and assimilation, as well as factors such as respiratory activity, gaseous diffusion and the solubility of dissolved gasses, which ultimately affect plant growth (Dogan et al 2010; Keerio et al., 2001).

High amount of mineral nitrogen in soil has negative effect on nodulation. Wide or narrow C:N ratio decreases nodule formation, therefore nitrogen fixation. If the C:N ratio is in expected ratio (15-30) nodulation and N_2-fixation regularly realizes. Inhibitory effect of nitrate causes the reduction of capillary roots development as well as preventing particular infection's strands. This effect is very similar to herbicides' effect. Many researches have shown that adequate nitrate, nitrite, ammonium and urea concentrations in soil causes to decrease the number of infections, to delay to the first formation of nodules, to decrease to the nodule number and weight. Temperature is the main factor affecting N_2-fixation; however, optimum temperature for N_2-fixation is depending on various soil properties. Optimum N_2-fixation temperature value is between 20-40 °C. Nodulation and nitrogen fixation in soybean is composed of between 20-30 °C. High soil temperature diminishes root growth as well as nodule formation. Furthermore, temperature changes affects to the competitive ability of Rhizobium/Bradyrhizobium species. Low temperatures decreased to nodule formation and N_2-fixation. However, N_2-fixation in natural legumes is not influenced extreme cold conditions (Bordeleau and Prevost, 1994).

Soil reaction (pH) is one of the most important factor influencing legume and Rhizobium symbiosis. A higher concentration of H^+ ions increases the solubility of Al, Mn and Fe, and higher amount of these elements may become toxic for rhizobium. *Sinorhizobium meliloti* and *Rhizobium galegae* are highly sensitive to acid pH and soluble Al when the critical soil pH is 4.8–5.0 (Bordeleau ve Prevost, 1994). *Rhizobium leguminosarum bv. trifolii* and *Rhizobium leguminosarum bv. Viciae* in comparison with alfalfa rhizobia are more tolerant to soil acidity. However, pH less than 4.6 inhibits their activity. Legumes and Rhizobium have form an efficient symbiosis and fix high amounts of biological nitrogen when soil pH is no less than 5.6–6.1. Soil acidification inhibited the root-hair infection process and nodulation. Optimum soil pH for nodulation and yield for soybean is between 6.2 and 6.8 (Lapinskas, 1998).

The results of a study indicate that *Rhizobium leguminosarum* bv. trifolii is widely distributed in slightly acid soils with pH_{KCl} 5.6–6.0. The average content of rhizobia was 540.0 • 10^3 cfu g^{-1} of soil. Less *Rhizobium leguminosarum* bv. viciae and significantly less *Sinorhizobium meliloti* and *Rhizobium galegae* were found. Rhizobium significantly declined in acid soils (pH_{KCl} 4.1–5.0). Most of biological nitrogen was fixed at soil pH_{KCl} 6.1–7.0. In this case, *Rhizobium galegae* accumulated 196 to 289 kg N ha^{-1} of nitrogen, whereas rhizobia of alfalfa and clover were less, and it depended on strain efficiency and soil pH. Soil liming had a positive effect on nitogenase activity in red clover. The soil liming ($CaCO_3$ rate 6.2 t ha^{-1}) in combination with inoculation have increased biological nitrogen fixation by red clover at 106 kg N ha^{-1}. Associative diazotrophes in non–legume rhizoplane have fixing the biological nitrogen too. The effective strains of *Rhizobium spp.*, *Agrobacter radiobacter* and *Arthrobacter mycorens* have made up an active association with barley, timothy and spring rape and accumulated 11.0 to 20.4 kg N ha^{-1} of biological nitrogen (Lapinskas, 2008).

Soil moisture can affect to nitrogen fixation both directly and indirectly. In low moisture condition in soil, nodule respiration decreases and nitrogen in nodule moves out slowly. This case is direct effect of low soil moisture. However in the same condition, nitrogen fixation decreased due to deterioration of generating photosynthesis units assimilate and in this case, N_2-fixation was affected indirectly.

Iron (Fe) and molybdenum (Mo) are located in structure of the Nitrogenase enzyme which is working with legumes for symbiotic nitrogen fixation (Fig. 6). Therefore, the amount of these nutrients in the soil and plant uptake affects the symbiotic N_2-fixation of legumes directly (Werner, 1987; Durrant, 2001).

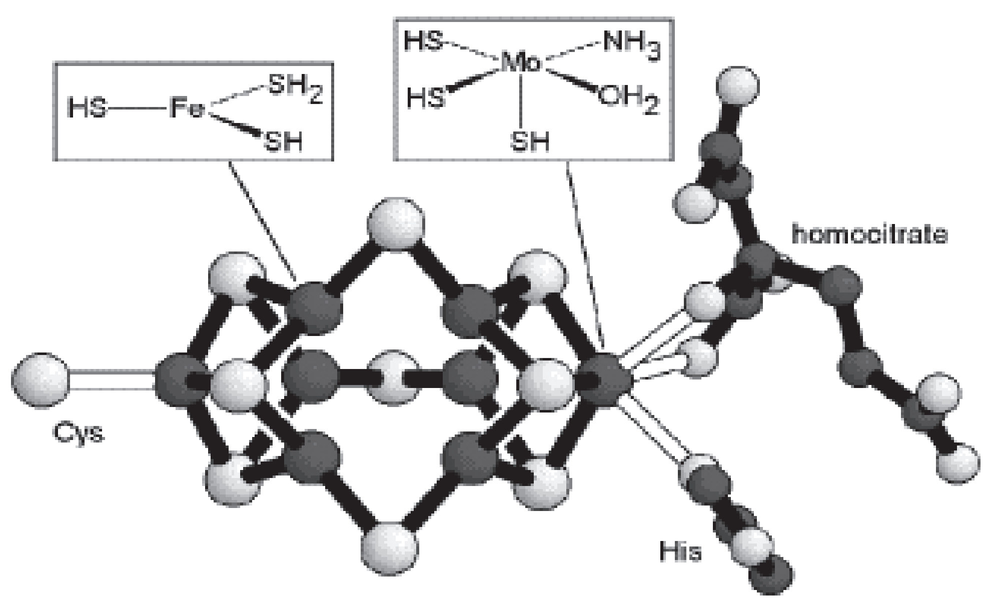

Fig. 6. Structure of nitrogenase enzyme (Durrant, 2001)

Nitrogen fixation in soybean is negatively affected by increasing salt contents of the soil. N_2-fixation of Rhizobium bacteria and their activities decreased in accordance with increasing soluble salt contents. Thus, increasing salt concentration in irrigation water was found to reduce a significant amount of grain and nodule weight in soybean (FAO, 1982).
According to many research it was determined to development of soybean was decreased in soil condition of 0.08% $CaCl_2$ and 1.5% $ZnSO_4$ (Anonymous, 1982). According to the results of many similar studies show that salt tolerance of rhizobium bacteria, optimum pH, antibiotic resistance and so on has revealed important differences (Gok and Martin, 1993).

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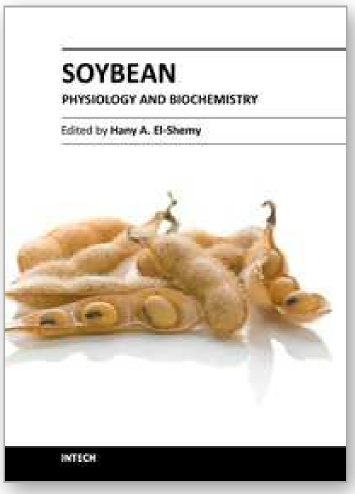

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