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Benefits and Limitations of *Rhizobium*-Legume Symbiosis and Nitrogen Fixation Under Libyan Saline Soil: Mini Review

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فرص استخدام وكفاءة الريزوبيم في تثبيت النيتروجين الجوي تكافليا مع البقوليات في الترب الملحية الليبية

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Abstract

Biological Nitrogen Fixation (BNF) donates to productivity both straight by increasing the production of legumes and indirectly by improving soil fertility. BNF is the cheapest and most environment-friendly procedure in which nitrogen-fixing bacteria (Rhizobia), interact with leguminous plants. Rhizobium is the most well-known species of the rhizobial group that acts as the primary symbiotic fixer of nitrogen, increased farming of legumes is important for the renewal of nutrient-deficient soils and providing required nutrients which important as food for human beings and animals. Some environmental factors such as salinity, which is become one of the severe difficulties in worldwide farming production. Where soil salinity limits the production of both feed and grain legumes.

Keywords: *Legume plants, N-fixation bacteria, Salt tolerances, Libyan soil.*

الملخص

يساهم تثبيت النيتروجين البيولوجي في الرفع من الإنتاجية الزراعية بشكل مباشر عن طريق زيادة إنتاج البقوليات وبشكل غير مباشر عن طريق تحسين خصوبة التربة. وتثبيت النيتروجين بيولوجيا هو الإجراء الأرخص والأكثر ملاءمة للبيئة، حيث تقوم البكتيريا المثبتة للنيتروجين (Rhizobia) بتثبيت النيتروجين الجوي تكافليا مع النباتات البقولية، حيث تقوم هذه البكتيريا باختزال النيتروجين الجوي الى أمونيا بواسطة أنزيم النتروجينيز نظرا لامتلاكها جينات تسمى Symbiotic Plasmid وهى المسؤولة عن إحداث العدوى في العقد البكتيرية ومن ثم تثبيت النيتروجين ، وكمية النيتروجين المتوفرة بالتثبيت مهمة جدا في زراعة البقوليات وزيادة نموها وانتاجيتها، وهذا التثبيت ضروري ايضا لتجديد خواص التربة وزيادة خصوبتها، وكذلك للمحافظة على النمو والنشاط الميكروبي في التربة، خاصة تلك التي تعاني من نقص في المغذيات كنتيجة للتأثير السلبي لبعض العوامل البيئية مثل زيادة الملوحة، والتي أصبحت واحدة من المشاكل المحددة في كمية الإنتاج الزراعي العالمي والليبي خاصة، حيث تحد ملوحة التربة من توفير أو زيادة في الانتاج الزراعي.

الكلمات الدالة: الريزوبيم، البقوليات، الملوحة، الترب الليبية.

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1. Introduction

Around 80% of Earth's atmosphere is nitrogen gas (N_2). Entirely all organisms on the planet use the ammonia (NH_3) form of nitrogen to build up very essentials compound in the life as amino acids, proteins, nucleic acids, and other nitrogen-containing components. BNF is the method that changes inactive N_2 into biologically suitable NH_3 form, this process is the greatest efficient way to quantity the large volumes of nitrogen needed by legumes to produce high-yielding harvests with a in height protein content (Tate,1995). On the other hand, this amount of nitrogen fixed by the legumes is influenced by several environmental conditions, soil nitrogen and phosphorous, compatibility of the rhizobia in the soil with the legume, and mycorrhiza colonization (Zahran,1999; and Nwoko and Sanginga, 1999). Worldwide, the consuming of fertilizer-N expanded from 8 to 17 $kg.ha^{-1}$ of agricultural land in the 15-year period from 1973 to 1988 (Zahran, 1999). The International Fertilizer Association (IFA) as cited in Heffer and Prud'homme (2016), expects that the demand world for N fertilizer would rise by 1.2% per annum among the base year (average of 2013/14 to 2015/16) and 2020/21 to reach 117.1 T_g N. Similarly, world N demand is predictable up by 1.3% p.a., from 101 T_g N in 2010 to 132 T_g N in 2030 (Heffer and Prud'homme, 2016). Where the annual consuming of fertilizer nutrient in terms of (N, P, and K), has increased also from 0.07 million metric tons (*MMT*) in 1951-52 to more than 25.95 *MMT* in 2016-17, and per h intake has increased from less than 1 kg in 1951-52 to the level of 130.8 kg nowadays (Bagal *et al.*, 2018). With the current skill for fertilizer production and the unproductive methods employed for fertilizer application, both the economic and ecological prices of fertilizer usage will eventually become expensive (Zahran, 1999). A widespread range of microorganisms that have the capacity to fix nitrogen, but Rhizobia only have a very great proportion on the nitrogen cycle (Tate, 1995; and Indge, 2000). Whatsoever the correct figure, legume symbioses provide at least 70 million tonnes of N per year, approximately half of this coming from the cool and warm temperature regions and the rest deriving from the tropics (Brockwell *et al*., 1995), not surprising that atmospheric N_2 fixed symbiotically by the relationship between rhizobium species and legumes represents a renewable foundation of N for crop growing (Wani *et al.*,1995; and Peoples *et al.*,1995). Standards estimated for several legume yields and pasture types are often imposing, frequently falling in the range of 200 to 300 *kg* of N/*hectare year*, and for example efforts of fixed N for alfalfa, red clover, pea, soybean, cowpea, and vetch were estimated to be about 65 to 335 kg of N/hectare.year (Zahran, 1999). Besides, the rhizobium symbiosis with legumes species is of special importance, making 50% of 175 million tons of total biological N fixation yearly worldwide, in other wards the biological processes account for about 60% for their total contribution to the nitrogen input (Ogutcu *et al.*, 2008). Biochemical nitrogen fertilization can considerably increase legume growth, but rhizobial inoculation characterizes as an economical and environmentally friendly alternative (Ogutcu *et al.*, 2008). Whereas the rate of N fixation differs and lay open to the cultivars and compatibility among the inoculated rhizobium strain and the host cultivar.

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The rhizobium species that making nodules in a hosts are specific only to this species or strain, and thus inoculation with active strains is advised in soils with no or weak bacterial being there. One of the best methods for sustainable agricultural includes the use of beneficial strains for plants, as they are able to promote plant growth by growing endophytically on plants. In recent years, the increasing worldwide worry regarding to the production of sufficient food to maintain the rising human population, thus has been reinforcing the importance of maintainable intensification of vegetable production.

This review attempts to cover how we deal with the soil salinity as an important problem facing farming production in several areas of Libyan agriculture, and limits legume efficiency, mainly when they depend on symbiotic N_2-fixation. Unfortunately, rhizobium and legumes related factors influencing symbiotic performance still undergoing studies and, poorly documented in home. Additionally, this review aims to explain the line for selection of the best rhizobium strain-legume variety mixture for maximum nitrogen fixation and harvest under saline soils.

2. The World of Legumes and Rhizobium

Legumes (*Fabaceae* or *Leguminosae*) is the third main angiosperm family, with more than 727 genera and 19,325 species, and include economically significant grain legumes, oilseed crops, shrubs, forage crops, and tropical or subtropical trees (Singh *et al*., 2007). Fabaceae are the next important food yield after cereals, they are bases of low-cost dietary and more safety vegetable proteins and minerals when compared with animal foodstuffs such as: egg, meat, and fish, and for other commercial applications (Singh *et al*., 2007; and Olunike, 2014). Africa has a huge array of indigenous legumes e.g. *Cajanus cajan*, *Lens esculenta*, *Glycine max*, *Phaseolus lunatus*, *Vicia faba and Vigna sinensis* (Singh *et al*., 2007), were legumes belonging to the genera *Astragalus*, *Hippocrepis*, *Hedysarum*, *Phaseolus*, and *Medicago* are distributed in Northern Africa (Lioi and Giovannetti, 1987; and Mahdhi *et al*., 2016). Despite the high number of these legume, only few of them have the capability to establish a symbiotic relationship through soil nitrogen-fixing bacteria (Allito *et al*., 2014; and Sagasti and Marino, 2015), legumes e.g. Clover, Common Bean, and Alfalfa (Mora *et al*., 2014). It is predictable about 88% of legume species that examined can form nitrogen fixing nodules with rhizobia, being responsible for up to 80% of the BNF that takes place in farming settings (de la Peña and Pueyo, 2012). Despite the fact that inoculating legumes with rhizobia can achieve considerable increases in nodulation, and biomass yield, but the truth is nitrogen fixation process controlled by the rhizobial strain, legume genotype and the environment factors (Zahran, 1999). While several rhizobium spp. normally occurs in soils but frequently fail to produce active nodulation (Jordan, 1982). However, the rhizobium bacteria are able to convert atmospheric N_2 into NH_4 in presence of the enzyme nitrogenase were the effective nodules occur as pink in color , thus due to a protein called leghemoglobin contains Fe and Mo, and it is responsible for binding oxygen to allows *Rhizobium* bacteria to live and to fix N_2. In addition, symbiosis is not only important for legume crops but also in nitrogen cycle, nitrogen

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comes from symbiosis process involving leguminous and Rhizobiaceae. The family of Rhizobiaceae presently involves six genera: *Rhizobium*, *Mesorhizobium*, *Sinorhizobium*, *Allorhizobium*, *Bradyrhizobium* and *Azorhizobium*, which are together referred to as *Rhizobia* (Datta *et al*., 2015). As cited in Yoon *et al*. (2010) the rhizobia or genus rhizobium was first proposed by Frank (1889) and its description was corrected by Young *et al*. (2001). However, it is classified in phylum *Proteobacteria*, and class *Alphaproteobacteria*, in order of *Rhizobiales*, and in family *Rhizobiaceae*. The genera of rhizobium have the following characteristics; obligate aerobes, gram negative, non-spore forming bacteria, chemoheterotrophs (require only simple organic compounds) and grown on a selective medium called yeast-mannitol agar (YMA), motile bacterium they can move using special thread-like structures called flagella, their shaped like short rods, 0.5-0.9 μm wide and 1.2-3.0 μm long, grown at 25-30$°C$ and optimum pH is 6-7. Overall, *Rhizobium spp.* are able to grow in microaerophilic conditions (Somasegaran and Hoben, 1994), and they are stimulated by root exudates, particularly the root exudates of their host legumes. Table (1) below gives some of the most important rhizobium spp. symbioses with their legumes.

Table 1. Rhizobium *spp*. and the cross-inoculation groups of legumes they modulate.

No	Rhizobium spp.	Legumes spp. examples
1	*Rhizobium etli*	Bean and Phaseolus
2	*R. galegae*	Galega
3	*R. gallicum*	Bean and Phaseolus
4	*R. giardinii*	Bean
5	*R. huakuii*	Astragalus
6	*R. huautlense*	Sesbania
7	*R. indigofera*	Indigofera
8	*R. leguminosarum*	Clovers; Peas and Vetch
9	*R. mongolense*	Medicago
10	*R. tropici*	Bean, Leucaena and Phaseolus

3. Soil Salinity Issues

Not surprising that the opening of 21st century is considered and marked by the global scarcity of water resources, environmental pollution and also increased salinization of soil and water (Shrivastava and Kumar, 2015). By 2050, it is evaluated that about 50% of the world's arable earthly will be influenced by salinity (Wang *et al*., 2003; and Bartels and Sunkar, 2005). At the present, salinity is a major problematic stressful crop production throughout the world: about 20% of urbane land in the world, and 33% of watered terrestrial, are salt-affected and deteriorated (Machado and Serralheiro, 2017). In addition, approximately 40% of the world's terrestrial surface can be considered as having possible salinity difficulties, and the greatest of these areas are confined to the tropics and Mediterranean regions (Monica *et al*., 2013). Bouksila (2011), recorded that 5-6 million hectares of arable land in the planet is destroyed yearly due to bad farming management and practices. Furthermore, 5% of this arable land was adversely affected by high level of salt, possibly due to bad agricultural management and practices, anywhere saline soils are common in areas of arid or semi-arid weather (Monica *et al*., 2013). Several environmental conditions (biotic and abiotic) are limiting aspects to the

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growth and productive of the N_2-fixing bacteria; include the host symbiont compatibility and the physicochemical situations of the soil. As the major serious threat faced by farming in arid and semi-arid areas is salinity, which counts as the most common issues affecting nitrogen fixation and symbiosis activity (Predeepa and Ravindran, 2010; and Monica *et al*., 2013), where arid and semiarid climates soils are usually have poor fertility; the rising of salinity and costs of chemical fertilizers and the growing environmental concerns. In compliance with USSL (1954), a saline soil is commonly defined as "a soil with an electrical conductivity of the soil solution extract (ECe) of 4 dS/m". However, desertification and limited freshwater resources resulting in decreasing farming yields and a deterioration in farmer livelihoods that often lead to the abandonment of earlier fertile lands and therefore to desertification. This has led to a histrionic reduction in the terrestrial of productive agricultural lands, in which saline conditions may limit the symbiosis in different ways includes: (a) decreasing the cell number (b) affecting survival and propagation of *Rhizobi*um spp. in the soil and rhizosphere, (c) preventing the infection process, (d) reducing photosynthesis, (e) creation of non-functional nodules with irregular structure, and (f) degradation of peribacteroid membrane (Predeepa and Ravindran, 2010; and Moussaid *et al*., 2015). In addition, the salinity can effect on crops by inhibit the plant's uptake significant nutrients, such nitrate and phosphorous, the salt reasons of ion cytotoxicity, and the osmotic stress (Chinnusamy and Zhu, 2004; and Monica *et al*., 2014), and finally low soil microbial activity (Yan *et al*., 2015). The basic solvable salts in soils are the cations Na^+ (sodium), Ca^{2+} (calcium), Mg^{2+} (magnesium) and K^+ (potassium), and the anions Cl^- (chloride), SO_4^{-2} (sulfate), HCO^-_3 (bicarbonate), CO_3^{-2} (carbonate) and NO_3^- (nitrate) (Tavakkoli *et al*., 2010; and Yan *et al*., 2015). However, there is a general consensus on the need to address the problem of soil salinity which developed an issue of main ecological and economic importance in several parts of the planet. Where most leguminous are more sensitive to salinity than their rhizobial fellow members, while the symbiosis process is more sensitive than rhizobia itself (Zahran, 1999). Nevertheless, some rhizobium species are found tolerant to salt stress, and promoting plant growth by developing specific devices to manage with salt stress, including the increase of mineral cations (e.g., K^+) and the intracellular creation of low-molecular-weight organic solutes such as; trehalose, proline, glycine betaine, and ectoines,. The previous compounds guard cells from desiccation and polyamines osmotic stress by improving the conformation of proteins and biological membranes (Mohammad *et al*., 1991.; Dong *et al*., 2017; and Santos and Costa 2002).

In fact some legumes are well at fixing nitrogen than others, but there is a general agreement that rhizobia are able to survive with salinity more than the host legume do, in contrast the difference in nodulation and nitrogen fixation frequently arise in a bacteria strain-legume that tolerant and survival under saline conditions. These bacteria are found in soil or in roots, it has been estimated that 1 *g* of soil may have a community of 10^9 microbes with Rhizobia (Datta *et al*., 2015). These days' rhizobium *spp*. has been broadly used to increase legume output in fields, by enhance both growth and nutrient uptake over modulation of hormone-linked phenomena in inoculated vegetation (Tate,1995).

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In the *Rhizobium*–legume symbiosis, the route of nitrogen fixation is powerfully related to the physiological status of the host plant. Consequently, an efficient rhizobial strain is not predictable to express its full volume for nitrogen fixation, if limiting causes impose limitations on the potency of the host legume. Numerous solutions have been suggested to managing this issue, in the emerging countries, the most hopeful solution for the near future has become the intensification of agricultural production through either increased cropping intensity and/or higher yields by introducing rhizobium-legume symbiosis as ideal solution to improving soil fertility (Mpepereki *et al.*, 2000), and facing salinity in the rehabilitation of arid lands. Therefore, several publications demonstrating that enhancing and inoculation of stress tolerant strains of rhizobium may improve the nodulation and nitrogen fixation capacity of legumes under harassed circumstances. For instance, the capability of legumes to grow and survive in saline conditions is enhanced when they are inoculated with salt tolerant strains of rhizobia (Zou *et al*., 1995; and Patil *et al*., 2014). The inoculation of legumes by salt-tolerant strains of *R. leguminosarum* bv. *trifolii* and *R. meliloti* was reported to enhancing nodulation and N content under salt stress up to 1% NaCl (El-Mokadem *et al*., 1991). A halotolerant strain EFB1 of *Rhizobium meliloti* was isolated from nodule of a *Melilotus* plant grown in a salt marsh, and shows their ability to grow at up to 500 mM NaCl (Lloret *et al.*, 1995). Also Elsheikh (1998) have concluded that rhizobia found to be useful and more salt tolerant than their host legumes. Other studies *e.g.*, Shamseldin and Werner in (2005) have isolated a high salt tolerance strains from Egyptian soils called *Rhizobium etli*, similarly his fellow countrymen Zahran (1999) have reported that *Rhizobium meliloti* more salt tolerant than their legume hosts, and can grow at 300 to 700 *mM* NaCl. In addition, three species of salt-tolerant rhizobia named as; *Rhizobium etli*, *Rhizobium gallicum* and *Sinorhizobium meliloti* have been isolated from Tunisian Oasis (Mnasri *et al*., 2007). Also Douka, *et al*. (2008) reported the same founding. Likewise, Ben Romdhane *et al*. (2008) found from their study that nodulation and yield of chickpea enhanced by inoculation with competitive and salt-tolerant rhizobia. Other study in United Arab Emirates have showed that the isolated rhizobia from desert soils were able to grow and successfully nodulate at high salt concentrations (Sharma *et al*., 2013). Others in Egypt have concluded that *R. leguminosarum* bv. *viciae* was able to survive at high salt concentrations at field conditions (Belal *et al*., 2013). In India Mandal (2014) has isolated five strains of *Rhizobium trifolii* HK, were they found to be resistances to 3% NaCl salt stresses. Moreover, Sobti *et al*. in (2015) have concluded from their study that the isolated rhizobia from the Algeria desert soils were able to grow and effectively nodulate even at high salt concentrations. Recently, Mahdhi *et al*. (2016) have found from 57 isolated bacteria from root nodules of two spontaneous legumes *Astragalus corrugatus* and *Hippocrepis areolata* were tolerated to 3% NaCl. Even so, the wide range of salinity levels characteristic of ecosystems where different legumes have been found to fix nitrogen better than others, this suggests that some legume species with specific rhizobial strains can be selected. For example: *Vicia faba*, *Phaseolus vulgaris* and *Glycine max*, were found to be more salt tolerant than other tested legumes (Cordovilla *et al*., 1999). In contrast, regarding tolerance to NaCl, TU in (1981) reported declines in growth of

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Rhizobium japonicum when NaCl % was increased from 0.2 to 0.87%. Also 30 isolated of root-nodulating bacteria they tested for their tolerance were found unable to grow at 2% NaCl, except one strain grew at 8% NaCl (Mohamed *et al*., 2000). Also a study by Thrall *et al*. (2008) have presents that there was sign for reduced strength of the plant–rhizobial mutualism as part of adaptation to salinity. Furthermore, isolates of rhizobia nodulaing from *Vigna Unguiculata* L. grown in arid area of Libya by Abdelnaby *et al*. (2015) showed different response to salinity ranging from sensitive, which unable to grow in 1% NaCl to resistant and grow at 2% NaCl or above. Interestingly introducing mycorrhizal to roots of legumes has been described to stimulate both nodulation and N_2 fixation, particularly in soils low in available phosphor where, phosphorus appears to be a very essential for both nodulation and N_2 fixation (Redecker *et al*., 1997).

4. Libyan Soil and Crops

Geographically, Libya has four different climates: (a) the Coastal Plains run along the Mediterranean Sea and have Mediterranean climate: warm, dry in Summers, and wet, cold in Winters; (a) the Northern Mountains, includes Jabal Nafusah in the West and Jabal al Akhdar in the East. they have the greater rainfall about 500 and 400 mm respectively and temperatures around 20 °C; (c) the center of Libya, where the desert, and (d) the Southern and Western Mountain part which recognized as a little annual rainfall region from 50 to 150 mm (Abagandura and Park, 2016). In general, the climate is characterized as either semi-arid or arid with very limited rainfall, and its volume and distribution varies from year to year where the agriculture in these area is primary dependent on irrigation. However, El-Asswad (1995) reported that the water level was decline and resulting seawater intrusion with the groundwater resources, which become almost unusable because of their high salinity, also he adding, from 1950 to 1990, the seawater interface advanced 1-2 km inland, and salinity increasing significantly from 150 ppm to around 1,000 ppm in the coastal aquifers. In fact, the majority of the Libyan soil is silty loam and very shallow and coarse, with low in organic matter content and water holding capacity, and about 22.9% of Libyan land is covered by salt flats, rock and dunes (Abagandura and Park, 2016). They added, however, that 95% of total land area in Libya is desert, whereas 4% is grassland, suitable for grazing animals, and 1% is forest, and the most agriculturally productive land limited to a strip abutting the Mediterranean Sea.

Salty soils are common in these areas where passage of soluble salts to the sea does not occur since of low rainfall, they are categorized by the occurrence of high levels of neutral salts in the surface layers' resultant from the capillary rise of water when evaporation surpasses precipitation (Abdelmoumen *et al*., 1999; Rao and Sharma, 1995; and Belal *et al*., 2013). As a result, most crops on the Libyan soil do not have sufficient soil nitrogen (Abagandura and Park, 2016), and the country now suffering from increases destroying agricultural land, with very large desert and arid climate. In Summer time some harvests are often irrigated with brackish or reused water, and as a consequence, salts gathering along the

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soil profile particularly if rainfalls in winter season are not sufficient to eliminate them from the rhizosphere (Abagandura and Park, 2016). Nonetheless, legumes are integral part of Libyan farming systems, and to meet with the consumers' requirements, over recent decades, our concern has been increased about the quantity and the quality of the legume production to feed, the constantly increasing human population has forced us to reinforce the importance of sustainable increase in crop productivity. The requirement for good agricultural practices is revitalizing the attention in BNF and Rhizobia-legumes symbiosis, predominantly those involving economically significant legume harvests in terms of nourishment and forage. Because all of that, more attention should be given to the legumes and rhizobia as they have the greatest quantitative influence on the nitrogen cycle and soil fertility. Refers to the above some rhizobium *spp*. and strains, which are reported as salt tolerant and efficient nitrogen fixers, have been cultivated (e.g. Bala *et al*., 1990; Zahran, 1999; and Sobti *et al*., 2015), this could be used as an alternative to achieve the establishment of symbiosis, where the selection of resistant strains is a significant choice, and can exist in Libyan soils. Overall, a very little work has been carried out on the Libyan legume and on salt tolerance strains of rhizobia, nevertheless the selection of salt-tolerant rhizobia from naturally soils is cheaper and more effective, and better able to nodulate the legume host below saline conditions. Interestingly the native rhizobial strains isolated by Keneni *et al*. (2010) was found more tolerance to higher salt concentrations of 5% NaCl than the exotic rhizobial strains did. Additionally, results from Ogutcu *et al*. (2008) have indicated that the chickpea was grow and survive in saline conditions when inoculated with *Rhizobium leguminosarum* bv. *ciceri* strains that isolated from wild chickpeas. Furthermore, Turkan and Demiral in (2009) have concluded that the efforts to upsurge salt tolerance of harvest plants is important to supply maintainable agriculture and it can improve crop productivity. Therefore, addition of organic materials such as vegetable remains or manures as nutrient sources may encourage rhizobia growth and activity, and could be an important strategy to ameliorate saline soils. At the same time, Rhizobia/legumes enhanced soil structure, erosion protection and better biological diversity.

5. Benefits and Limitations

This paper recognizes the role of biological nitrogen fixation as an unpolluted and more cost-effective way to increase Libyan soil fertility and adaptation to saline condition. In detail, the existence of *Rhizobium*- legume symbioses, which are capable to fix appreciable sum of N_2 under arid conditions is attractive. However, these symbioses signify the best source of the "ideal" fertilizer in arid and semi-arid regions in the country which known as having a high level of salinity and low fertility. On the other hand, under saline conditions the response to rhizobium inoculation, was found to be affected by soil textures, from no response to a highly significant response (Elsheikh,1998). Not surprising that under salt stress some plants differs from others in their tolerances, Pea plants (*Pisum sativum*) showed higher levels of nodule mass and nitrogen fixation than faba bean (*Vicia faba*) plants (Cordovilla *et al*.,1999). Therefore, it is very important to promoting rhizobial growth to contribute by selecting high salt-tolerant strains under salt stress, and understanding the fixation process of nitrogen by

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legumes, and how they change within the soil, this will help us to meet with the N potential of our crop production and will help minimize the possibility to reducing the soil salinity. Several recent studies have used molecular techniques e.g. (Shamseldin *et al*., 2008; Zahran *et al*., 2013; Moghaddam *et al*., 2018) including; Plasmid profile, DNA extraction, Polymerase chain reaction (PCR): Metabolomics, Proteomics, and Transcriptomics, to define new genes, proteins, and metabolites involved in a nonliving condition in legumes (abiotic factors). As Libya known as a emerging countries should focus on providing adequate food to their people, which requires both increasing yields and maintain soil fertility. In this way, hope this review assists the Libyan researchers in becoming a more active to introduce the superior rhizobium strains, to produce industrial and saleable biological fertilizer, which is still poorly understood, where, the government through Ministry of Agriculture should recognize that this kind of work must be supported to maintain our food supply.

6. Conclusion

From the above, it can be without difficulty concluded that, nitrogen fixation efficiency depends on rhizobium strain, plant host, salt (a biotic) factor, soil and their interaction. The salt tolerant rhizobium-legume relations well have good possibility for improving the fertility of Libyan saline soils, where introducing tolerant salinity crops such as: barley, oat and species of salt-tolerant olive trees is needed. Whereas a successful management of symbiotic association between leguminous vegetation and their bacterial endosymbionts essentials to improves yields in saline areas. While screening and application of effective tolerance rhizobial strains by using genetic engineering as today an important technological tool will lead to improve *Rhizobium-legume* resistance to salt stress. Further investigation should be carried out about the diversity to select salt tolerant strains of rhizobium bacteria. However, we should take in our account that our emphasis is to select the effective *Rhizobium* strain to achieve maximum benefits from the new technology. It is hereby recommended that there is need to understanding the effects of salinity on symbiotic nitrogen fixation other than the survival of the Rhizobium *spp*. Further advanced study by using molecular tools techniques for identification of rhizobium isolates within host leguminous crop are required.

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