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Original Article

Stress Factors Affecting Symbiosis Activity and Nitrogen Fixation by *Rhizobium* Cultured *in vitro*

NISTE Monica*, Roxana VIDICAN, Rodica POP, Ioan ROTAR

University of Agricultural Sciences and Veterinary Medicine Cluj - Napoca, Mănăştur St., No. 3 - 5, 400372 Cluj-Napoca, Romania

Abstract

Rhizobium (family *Rhizobiaceae*) is a group of symbiotic bacteria which fix atmospheric nitrogen throughout nodule formations, can be found in soil or in roots of legumes. This process transforms atmospheric nitrogen to ammonia, nitrate and nitrogen dioxide. Within the soil, rhizobia frequently encounter various stresses that affect their growth, their initial steps of symbiosis and the capability of nitrogen fixation. Biological nitrogen fixation is a critical and key process in sustainable land management, nitrogen being the nutrient that limits crop production in a high level. Biotic and abiotic stresses impose a major threat to agriculture and symbiotic nitrogen fixation is dependent on host cultivar and rhizobia, but as well may be limited by pedoclimatic factors. The most common factors affecting nitrogen fixation and symbiosis activity are salinity, drought and temperature.

Keywords: *Rhizobium*, symbiosis, nitrogen fixation, salinity, temperature, drought.

1. Introduction

Root nodule bacteria, known as rhizobia, form symbiosis with different leguminous plants. Nitrogen (N) occurs in the atmosphere as N_2, a form that is not useable by vascular plants. Nitrogen must first be fixed, or reduced, to ammonia (NH_4^+). Symbiotic nitrogen fixation involves different hosts and microsymbionts between legumes and bacteria belonging to the genera *Rhizobium, Bradyrhizobium* and *Azorhizobium* [3]. First, binding of bacteria to the wall of root hairs involves close contact, mandatory, necessary for induction of morphological response ensuring symbiosis installation [10]. The symbiotic bacteria, differentiated into bacteroids and surrounded by a peribacteroid membrane (that isolates them from the host cytoplasm), fix nitrogen inside the plant cells [7].

* Corresponding author.
Tel.: 0040264596384; Fax: 004026359379
e-mail: monikniste@yahoo.com

Interaction of *Rhizobium*/vegetables involves, firstly, the existence of a compatibility phenomenon between two partners, thus limiting structures are activated host infection, accumulation of inhibitory substances or signs of hypersensitivity which effectively prevents the spread of infection. *Rhizobium* responds to certain flavonoids secreted by the plant and in turn it synthesizes specific factors capable of inducing the formation of the nodule (fig. 1). Through an infection the bacteria enters into the root of the plant and afterwards infiltrates the host cell cytoplasm.

Multiplication and hypertrophy due to abnormal but limited simulated cortical cells infected with *Rhizobium* nodule occur, they are a highly differentiated and organized plant body, providing the necessary protection requirements N_2 fixation and other metabolic processes of the two symbionts [10].

Biological nitrogen fixation is a critical and key process in sustainable land management, nitrogen being the nutrient that limits crop production in a high level [3].

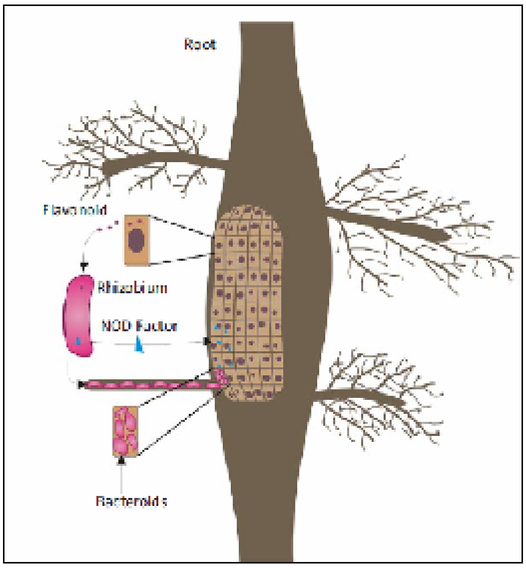

Figure 1. Nodulation process
(Source: www.yasni.it)

Biotic and abiotic stresses impose a major threat to agriculture and symbiotic nitrogen fixation is dependent on host cultivar and rhizobia, but as well may be limited by pedoclimatic factors.

2.Stress factors affecting symbiosis and nitrogen fixation

In the entire world plant productivity is limited by some environmental factors most common being: salinity drought, nutrient imbalances (including mineral toxicities) and extremes temperatures.

Nearly 40% of the world's land surface can be categorized as having potential salinity problems, most of these areas are confined to the tropics and Mediterranean regions [12]. Worldwide 100 million ha or 5% of the arable land is adversely affected by high level of salt [2] because of this condition many studies contemplate the effect of salt on the growth of *Rhizobium*. Saline soils are common in regions of arid or semi-arid climate where potential evapo-transpiration exceeds annual precipitation and is a serious risk to agriculture.

They are characterized by the presence of high levels of neutral salts in the surface layers resulting from the capillary rise of water when evaporation exceeds precipitation [3].

The salinity response of legumes, like that of most cultivated crops, varies greatly and depends on climatic conditions, soil properties, and growth stages [12]. Salinity may occur when there is irregular irrigation, inadequate drainage, wrong fertilizer application and it extremely increases in protected cultivation [2].

Most leguminous plants require a neutral or slightly acidic soil for growth, especially when they depend on symbiotic N_2 fixation, hosts being more sensitive to salinity than their rhizobial counterparts. The symbiosis is more sensitive to salt stress than free-living rhizobia [12]. Different steps of the symbiotic interaction as well as nodule development and metabolism are affected by salt stress and this process leads to a reduction in nodule number [9].

The predominant salts are usually sulfates and chlorides of sodium, calcium and sometimes magnesium and small quantities of carbonates and bicarbonates are often present. These soils are only moderately alkaline, with pH about 8, and their agricultural use usually demands irrigation [3]. Increases in the salinity of soils or water supplies used for irrigation result in decreased productivity of most crop plants and lead to marked changes in the growth pattern of plants. Increasing salt concentrations may have a detrimental effect on soil microbial populations as a result of direct toxicity as well as through osmotic stress [12].

Plants living on salt soil may show some deficiencies like affections in every aspect of plant physiology, biochemical and genetic mechanisms. This processes occurs throughout decreasing the amount of soil water available and increasing the concentration of sodium (Na) and chloride (Cl). Survival and proliferation of *Rhizobium* spp. in soil and rhizosphere is affected and inhibits the infection process, directly affecting root nodule function, plant growth and demand for nitrogen [14]. Other effects of this process are: disruption of photosynthesis and photorespiration and lack of mineral nutrients. The general effect of soil salinity on plants is called a physiological drought effect [2].

The response and adaptation of rhizobia to salt stress is a complex phenomenon implicating many physiological and biochemical processes that notably affect rhizobial colonization of roots and early infection events [7].

One of the major constraints of salt stress is nutrient imbalance, caused by the loss of control on nutrient uptake and/or transport to the shoot leading to ion deficiencies. Major cations (Na^+, Ca^{2+}, Mg^{2+}, K^+) and anions (Cl^-, SO_4^{2-}, HCO^{3-} CO_3^{2-} and NO_3^-) accumulate in soil in high quantity. Salt injury is due to Na^+ or Cl^- (or both) accumulating in transpiring leaves to excessive levels, exceeding the ability of the cells to distribute these ions in the vacuole. Ions then expand rapidly in the cytoplasm and inhibit enzyme activity or they build up in the cell walls and dehydrate the cell [6].

Some studies suggest the need to select plant genotypes that are tolerant to salt stress and then match them with the salt-tolerant and effective strain of rhizobia. In fact, the best results for symbiotic N_2fixation under salt stress are obtained if both symbiotic partners and all the different steps in their interaction (nodule formation, activity, etc.) resist such stress [12].

Nearly all areas of the world are subjected to rain-free periods for sufficient duration results in extensive drying of soil, and the rhizobia that must survive in these soils in order to bring about nitrogen fixation in the succeeding crop may thereby become too few in number to cause extensive nodulation [3].

The survival of rhizobia in soil is more affect by high temperatures then by low temperature, because this can be deleterious [3], affecting both partners of symbiosis and all steps in the development of an efficient nitrogen fixation. Temperature affects root hair infection, bacteroid differentiation, nodule structure, and the functioning of the legume root nodule [12].

In addition, the relative activity of the rhizobia is altered by temperature, so that a bacterium that is highly effective at one temperature is less active at different temperatures. For these reasons, greater nitrogen gains probably can be achieved by improvements in the heat resistance of the symbiosis. The optimum temperatures for growth in culture varies among strains and species, values between 27 - 39°C have been noted. The maximum temperatures are generally 35 - 39°C, but proliferation may take place up to 42°C [3].

Rhizobial survival in soil exposed to high temperature is greater in soil aggregates than in non-aggregated soil and is favored by dry rather than moist conditions [12].

The occurrence of rhizobial populations in desert soils and the effective nodulation of legumes growing there in emphasize the fact that rhizobia can exist in soils with limiting moisture levels. Rhizobial growth and symbiosis process is affected by drought conditions. Viable strains of *Rhizobium* usually cannot tolerate or function under high levels of osmotic stress caused by drought. N_2-fixing legumes are especially sensitive to water deficit and other environmental stresses, with drought being one of the major environmental factors affecting plant productivity [12].

The common effect of drought on rhizobia results in osmotic stress, which leads to changes in rhizobia morphology [4], persistence and survival in soil, root-hair colonization and infection [12] as well as dehydration of cells.

Soil moisture deficit has a marked effect on N_2 fixation as nodules initiation, growth and training are more sensitive to moisture than roots in general. Nodules and N_2 fixation response to water factor depends on the stage of plant development. Water stress during growth has a direct effect on the development of nodules than in other stages and the possibility of recovery is almost impossible.

The effort to develop stress tolerant plants is very important to increase crop productivity. Induction of genetic variability can be achieved by exposing cultured cells or tissues to action of physical or chemical mutagens. This technique can operate under controlled conditions with limited space and time. In recent years, *in vitro* selections based on tissue culture appear an appropriate and profitable tool for developing stress-tolerant plants.

Plants tolerant to both biotic and abiotic stresses can be acquired by applying the selecting agents such as NaCl (for salt tolerance), PEG or mannitol (for drought tolerance). The selection of somaclonal variations used in regenerated plants may be genetically stable and can be integrated in crop improvement [5].

Since the first report in *Nicotiana sylvestris*, many attempts have been made to produce salt tolerant plants using *in vitro* techniques [5]. Research into the genetic engineering of rhizobia is aiming for the development of salt-tolerant strains that can tolerate high levels of salinity in the soils and would be a practical solution for such problems [11]. Increasing salt concentration may have detrimental effects on rhizobial population. Rhizobia contrary to their host legumes, can survive in the presence of high levels of salt.

Rhizobial inoculation increases nodule biomass thus encourages sustainable environmental friendly agriculture by responding perfectly in biological nitrogen fixation [1].

Two types of selection methods has been suggested: stepwise long-term treatment, in which cultures are exposed to stress with gradual increase in concentrations of selecting agent and shock treatment, in which cultures are directly subjected to a shock of high concentration and only the tolerate ones will survive [8].

During past years, *in vitro* selection for cells exhibiting increased tolerance to water or drought stress has been reported. *In vitro* culture may be used to obtain drought-tolerant plants assuming that there is a correlation between cellular and *in vivo* plant responses [5]. *Rhizobium* growth and survival are generally more tolerant *in vitro* to high osmotic pressures than their respective host legumes [9].

Further progress in improving crop resistance to abiotic stress will depend on new gene discoveries, leading to a complete understanding of stress protection, efficient transformation technologies applicable to a wider range of species and the evaluation of transgenic materials [13].

3. Conclusions

Environmental factors affect symbiotic process and nitrogen fixation by *Rhizobium* in soil.

There have been discovered some symbiotic systems that are tolerant to certain stress factors like: salinity, drought, extreme temperature, metal toxicity. This relationship can ensure successful molecular nitrogen fixation under less favorable factors.

Under poor conditions *Rhizobium/l*egumes symbiosis is very important because it may be the only way to fix nitrogen.

In the past century the tremendous progress have been made in understanding the process of symbiosis and nitrogen fixation, and using genetic engineering will lead to improve the plant resistance to environmental factors.

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