International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Abiotic Stress in Leguminous Crop Plants

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Abstract: ***One of the most severe and widespread problems facing the agricultural industry is the degradation of soil quality due to desiccation and acidity and it would harsh environmental conditions and have a dramatic impact on the endogenous soil bacteria, the rhizobia. These bacteria induce formation of nodules on legumes, in which atmospheric nitrogen is fixed and supplied to the plant, enhancing growth under nitrogen limiting conditions.Desiccation and acidity negatively affect such interactions by limiting nodulation processes and nitrogen fixation*** [31]***. Thus invitro evaluation of strains under stress might be a useful method in finding rhizobia isolates adapted to different environments where extreme temperatures and pH limit symbiotic nitrogen fixation. Hence, the review article discusses the rhizobia strains tolerant to high temperature and acidic stress conditions in leguminous crop plants.***

Keywords: Proteobacteria Rhizobia, extreme temperatures, pH, nodulation, nitrogen fixation

1. Introduction

Abiotic stresses like drought, low temperature and high salinity are environmental factors that dramatically limit plant growth and crop productivity [2], has been estimated that 10 per cent of the arable land can be classified under the non-stress category, which implies that the crops grown under 90 per cent of arable land experience different environmental stresses, singly or in combination. The prediction is that the water deficits would continue to be the major single abiotic factor likely to affect crop yields globally.

The Semi-Arid Tropics (SAT) are prone to hot summers and erratic rainfall having the season length of less than 100 days. Agriculture in these environments means growing marginal crops on marginal lands with marginal resources. The productivity of crops in these regions is severely affected by drought besides several biotic constraints [26]. Some of the most important crop of this region includes groundnut, chickpea, pigeon pea, sorghum, and pearl millet that are the mandated crops of ICRISAT and are the main staple food for nearly one billion people in the SAT. Amongst these groundnuts, chickpea and pigeon pea are legumes that are significant groups of agriculturally important crops and have been the subject of widespread efforts to improve desirable traits. Though, quantitative estimates of yield losses due to drought in tropics are scarce and imprecise, according to some predictions [27], the productivity could be increased over the present levels in groundnut, chickpea and pigeonpea by 29, 49 and 57 per cent respectively, if water was not limiting. However in rainfed agriculture, there was an option to increase under irrigation and the only option is to improve the realizable yields under water deficit conditions. Although drought management has been an option to increase the yield and realized that the genetic improvement of drought tolerance is more rewarding [26]; [24]. Infact, the genetic engineering approaches have been shown to be relatively fast and precise means of achieving improved stress tolerance.

2. Temperature

Temperature is one of the most important factors affecting any organism [8]. When cells are exposed to high temperature or any other environmental perturbation such as UV irradiation, nalidixic acid [14] or ethanol [20], a group of proteins called heat shock proteins (Hsps) are rapidly synthesized [22].

Nodule functioning in common beans (*Phaseolus* spp.)is optimal between 25 and 30°C and is hampered by root temperaturesbetween 30 and 33°C. Nodulation and symbiotic nitrogenfixation depend on the nodulating strain in addition to the plantcultivar. Temperature affects root hair infection, bacteroid differentiation, nodule structure, and the functioningof the legume root nodule.

[19] studies showed evidence that the influence of root temperature on nodulation and dinitrogen fixation by *Bradyrhizobium* sp. forming nodules on peanut plants. Compared to 25ºC, temperatures of 30 and 35ºC have been shown to reduce nitrogenase activity of peanut root nodules, but nitrogenase activity was measured only by *invitro* acetylene reduction rates using detopped bare root systems.

The cultivated peanut (*Arachis hypogea* L.) is primarily a crop of the tropical and subtropical areas where surface soil temperatures above 35 ºC are not uncommon. It has been shown for other plants grown in tropical and subtropical 35 to 45ºC are detrimental to nodule formation or dinitrogen fixation. Effect of room temperature on nodule formation and dinitrogen fixation of legumes is modified by the strain of *Rhizobium* [1]

[17] found that the heat-tolerant rhizobia formed less effective symbiosiswith their legume hosts. Several heat-tolerant N_2-fixingbean-nodulating*Rhizobium* strains which grow at 40°C have beenisolated.

[10] inferred that the optimum temperature range for rhizobial growth in culture was 28 to 31°C, and many were unable to grow at 37°C. However, 90 per cent of cowpea *Rhizobium* strains obtained fromthe hot, dry environment of the Sahel Savannah grew well at 40°C.Heat treatment of

R. phaseoli at 35 and 37°C resulted in mutant strains lacking a plasmid DNA implicated in the synthesis of melanin and is related to the loss of symbiotic properties of these bacteria .Screening of *R. leguminosarum*bv. *phaseoli* showed that some strains were able to nodulate *Phaseolus vulgaris* at high temperatures (35 and38°C) but that the nodules formed at high temperatures were ineffectiveand plants did not accumulate N in shoots.

[16] reported that the high soil temperatures in tropical and subtropical areas are a major problem for biological nitrogen fixation of legume crops. High root temperatures strongly affect bacterial infectionand N_2 fixation in several legume species including soybean, guar, peanut, cowpea, and beans.Critical temperatures for N_2 fixation are 30°C for clover and pea and range between 35 and 40°C for soybean, guar, peanut, and cowpea.

[29] reported that the UV mutant MB 1 was exposed to a range of temperature *viz*., 35, 40, 45 and 50°C and pH of 5.5 and 6.0 for 24 – 96 h of incubation to know their tolerance limit and the results showed that the population (8.21 to 8.36 log $_{10}$cfu ml^{-1}) of UV mutant MB1 was increased to significant level for 24 – 72 h of incubation at 35°C and pH 5.5. When the temperature increased from 35 to 45°C, there was a gradual decline in the population level (8.21 to 7.72 log $_{10}$cfu ml^{-1}) at pH 5.5. At 50°C, the population was increased (7.30 log $_{10}$cfu ml^{-1} to 7.60 log $_{10}$cfu ml^{-1}) upto 48 h of incubation beyond which the multiplication of rhizobia reduced further. The interaction between the hours of incubation, temperature and pH had significant effect on the survival of rhizobial mutants.

3. Soil Acidity and Alkalinity

Soil acidity can adversely affect the formation of symbiotic association between *Rhizobium* species and their host legume plants. [18] reported that the acid sensitive strains of *R. meliloti* failed to maintain a pH gradient in acid conditions and were, thus unable to control intracellular pH.

Repeated subculturing of *Rhizobiumleguminosarum*bv*trifoli* and *Bradyrhizobium* strains on stress media or progressive culturing on media of decreasing pH failed to increase levels of tolerance to low pH and did not give rise to spontaneous variants with increased levels of acid tolerance. The phase of the legume *Rhizobium* symbiosis was affected due to i) rhizobial survival in the soil and growth in the rhizosphere ii) infection and nodule establishment iii) nodule function iv) growth of the host plant. *Rhizobium* sp. are known to differ in their tolerance of soil acidity; the slow growing *Bradyrhizobium* strains being generally more acid tolerant than the fast growing species, especially *Rhizobiummeliloti*. Hydrogen ion activity is a major factor restricting the survival and growth of rhizobia in soil.

[15] found that most of the leguminous plants require a neutral or slightly acid soil pH for growth and nodulation; problems are to be expected once the pH falls below 5.5. Soil acidity adversely affected the survival, growth and nitrogen fixation of microorganisms, while nutritional disorders affected legume *Rhizobium* symbiosis.

[9] reported that the acid tolerant strains of *Rhizobium meliloti* have recently been used to establish *Medicago polymormpha* based pastures on more than 350,000 ha of acidic soils in Western Australia which were previously considered too acidic to support the growth of this legume. Inoculant strains of *Rhizobium leguminosarum* bv *trifoli* (WU95 and TA1) have been shown to be sensitive to low pH as reflected by their poor growth in acidified laboratory media. However strains of *Rhizobium leguminosarum* bv *trifoli* with increased acid tolerance had been isolated but such strains commonly exhibit poor levels of symbiotic effectiveness in association with *Trifolium subterraneum*. Transfer of acid tolerance from the inoculant strains of *Rhizobium leguminosarum* bv *trifoli* was done.

[21] suggested that the acid tolerant strains of *Rhizobium meliloti* can more readily generate a pH gradient when grown in acid conditions and can be subsequently maintain a more constant internal pH.

[7] observed the development of inoculant strains of *Rhizobium leguminosarum*bv*trifoli* with enhanced capacity for growth at low pH may provide a means by which nodulation and growth of *Trifolium*spp. in acid soils can be improved. pH stress also limits nodulation and nitrogen fixation. Legume species vary markedly in their tolerance to Al $^{3+}$and Mn^{2+}, with some plants being significantly more strongly affected by these ions than are the rhizobia.

4. Effect of Severe Conditions on Nodulation and Nitrogen Fixation

Nodulation and nitrogen fixation in legume/*Rhizobium* associations are adversely affected by salinity and drought, which can preclude legume establishment and growth or reduce crop yield. Moreover, commercial strains of *Rhizobium* usually cannot tolerate or function under high levels of osmotic stress caused by salinity and drought. e.g., salt in peat used as a carrier reduced the viability of *Rhizobiumtrifoli* in legume inoculants produced in Australia. The microbial symbionts tolerated higher levels of salinity than the host plant and it should not give the impression that the crop performance will automatically improve without concern for the host plant when inoculated with salt tolerant *Rhizobium*.

[23] suggested that the nitrogenase activity was not affected in cowpea nodules when the temperature was increased from 15°C to 35°C but decreased severely at temperatures above 38°C.

[5] and [31] reported that rhizobia are soil bacteria which can able to infect the roots of leguminous plants, promoting the formation of nitrogen fixing nodules. The symbiosis between rhizobia and legumes is the most important biological mechanism for providing nitrogen to the soil or plant system and thus reducing the need of the plant crops for chemical fertilizers.

5. Soil Moisture Deficiency

One of the immediate responses of rhizobia to water stress (low water potential) concerns the morphological changes. Mesquite*Rhizobium* and *R. meliloti* showed irregular morphologyat low water potential. The modification of rhizobial cells bywater stress will eventually lead to a reduction in infectionand nodulation of legumes.

A favourable rhizosphere environmentis vital to legume - *Rhizobium* interaction; however, the magnitudeof the stress effects and the rate of inhibition of the symbiosisusually depend on the phase of growth and development, as wellas the severity of the stress. For example, mild water stressreduces only the number of nodules formed on roots of soybean, while moderate and severe water stress reduces both the numberand size of nodules [28].

Symbiotic N_2 fixation of legumes is also highly sensitive to soil water deficiency. Soilmoisture deficiency has a pronounced effect on N_2 fixation becausenodule initiation, growth, and activity are all more sensitiveto water stress than are general root and shoot metabolism. The response of nodulation and N_2 fixation to water stress depends on the growth stage of the plants. It was found that waterstress imposed during vegetative growth was more detrimental tonodulation and nitrogen fixation than that imposed during thereproduction. There was little chance for recoveryfrom water stress in the reproductive stage. Nodule P concentrationsand P use efficiency declined linearly with soil and root watercontent during the harvest period of soybean-*Bradyrhizobium* symbiosis [4].

[12] reported that the low water content in soil was suggestedto be involved in the lack of success of soybean inoculation insoils with a high indigenous population of *R. japonicum*.Further, a reduction in the soil moisture from 5.5 to 3.5 per cent significantlydecreased the number of infection threads formed inside root hairsand completely inhibited the nodulation of *T. subterraneum*.Similarly, water deficit simulated with polyethylene glycolsignificantly reduced infection thread formation and nodulationof *Viciafaba* plants.

[3] showed that several mechanisms have been suggested to explain the varied physiological responses of several legumes to water stress. Thelegumes with a high tolerance to water stress usually exhibitosmotic adjustment; this adjustment is partly accounted for bychanging cell turgor and by accumulation of some osmotically activesolutes. [25] found that N derived from N_2 fixation was decreased by about 26 per cent as a result of waterdeficiency when measured by the acetylene reductionassay.
[29] reported that the effect ofthree soil moisture potential (-0.03 -1.00 and -1.5 Mpa) were taken into consideration for the study. Among the five isolates tested at -0.03 Mpa, BlackgramBcp1 isolates had significantly maximum growth (8.75 $\log_{10}$cfu ml^{-1}) and it was gradually decreased (8.20 $\log_{10}$cfu ml^{-1}) after 36 th day of inoculation. Comparatively at -1.00 Mpa, the growth (8.60 $\log_{10}$cfu ml^{-1}) was decreased and reduced further (7.40 $\log_{10}$cfu ml^{-1}) at -1.5 Mpa followed byGreengram Gcp1, Groundnut Gncp1, Soybean Scp1 and Cowpea Ccp1 isolates.

Potassium is known to improvethe resistance of plants to environmental stress. The presence of 0.8 or 0.3 mM K^+ allowed nodulation and subsequent nitrogen fixation of *V. faba*and *P. vulgaris* under a high-water regimen (field capacity to 25% depletion). It was also shown that the symbiotic system in these legumes is less tolerant to limiting K supply than are theplants themselves. Species of legumes vary in the type and quantityof the organic solutes which accumulate intracellularly in leguminous plants under water stress. This could be a criterion for selecting drought-tolerant legume-*Rhizobium* symbioses that are able to adapt to arid climates.

[29] reported *that the Rhizobium* Bcp1 isolates grown at pH 5.5 had maximum growth (9.00 $\log_{10}$cfu ml^{-1}) at ‘0’µM aluminium concentration significantly after 15 days of incubation. When the aluminium concentration was increased from 0 to 50 µM, the population decreased. *Rhizobium* Bcp2 isolates grown at pH 6.0 showed the maximum growth (9.15 $\log_{10}$cfu ml^{-1}) at ‘0’µM aluminium concentration, compared to *Rhizobium* Bcp1 isolates.

At ‘0’µM aluminium concentration, the Greengram*Rhizobium* Gcp*1* isolates grown at pH 5.5 recorded the maximum growth (7.50 $\log_{10}$cfu ml^{-1}) and it was reduced to 7.41 $\log_{10}$cfu ml^{-1}at 25 µM and 7.35 $\log_{10}$cfu ml^{-1} at 50 µM aluminium concentration.

Greengram*Rhizobium* Gcp2 isolates grown at pH 6.0 showed the maximum growth (9.20 $\log_{10}$cfu ml^{-1}) at ‘0’µM aluminium concentration. Then it was reduced to 9.10 $\log_{10}$cfu ml^{-1} at 25 µM and 9.07 $\log_{10}$cfu ml^{-1} at 50 µM aluminium concentration.

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