Studies on symbiotic nitrogen fixation under soil stress cond

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Until perhaps 10 years, studies of environmental stress and thelegume-Rhizobium or Bradyrhizobium symbiosis were restricted todefining the problem, and to using physical or chemical amendments toovercome it, i.e., mulching to reduce soil temperature or liming toameliorate soil acidity. The identification of bacterial strains and in some cases host cultivC;lfs that are tolerant to these stresses opens the way for alternate, lower cost solutions to these problems. c' Therefore, in the present study, the ability of2S local and foreign Rhizobium and Bradyrhizobium strains represent R. meliloti (5 strains),R, leguminosarum bv. viceae (5 strains), R leg!lminosarum bv. trifolii(2 strains), Bradyrhizobium sp. nodulating peanut (5 strains) and.Bradyrhizobium . japonicum. (5 strains) to tolerate increasedconcentrations of NaCI, high temperature, high levels of nitrogen, high pH values and desiccation was investigated. Rhizobial strains tolerant to the above mentioned stress conditions were selected and the symbiotic performance of the obtained strains was evaluated as well. Results are summarized as follows :1- Effect of NaCI salinity on growth and survival of rhizobia:1.AU tested strains were able to tolerate NaCl concentration up to O.1°/0 However, tolerance of NaCI salinization differed according torhizobial spp. and strains. In general, R. meliloti and Kleguminosarum bv. phseoli were found to be more sensitive to NaClsalinity . 2.There was a great variation in the response of the tested strains tosodium chloride regimes. All strains were sensitive to higherconcentration of NaCI(S%). The growth of some strains wascompletely inhi~ited at 20/0 NaCI. Some ,strains were sensitive to even20g IL NaCl. 1. Two Rhizobium l~guminosa,.um bv. viceae ARC 200Fand ICARDA 441 were tolerant to 10 and 40 gIL NaCl, the two strains wereidentified as low and high tolerance to NaCl salinity respectively ..2- Growth and survival res onse of rhizobia to increasin tem erature:I 1.The most of the rhizobial strains-tested showed optimum growth at 30-350C , then higher temperature resulted in gradual decreases inrhizobial numbers. Susceptibility to higher temperature of 50 ~Ciscommon among the rhizobia! species and strains under investigation.2. The decline in, the number of surviving strains was especiallynoticeable between 40 and 45°C. This behavior depends on therhizobial sp. and ·strain.3. In general, fourteen strains were found to tolerate temperature up to 450C. such as Rhizobium meliloii (ARC 1, ARC 2, and A 2), Rhizobiumleguminosarum bv. viceae (ARC 200 F and ARC 202F), Rhizobiumle~minosarum . bv. trifolii (TAL), Rhizobium lflEU_mirlOsaromby.phaseoli tARC 302 and UMR), peanut Bradyrhizobium (619, 3339and 601) and firadyrhizobium jqponicum (138, USDA 110 and ARC4. Three strains, however, were found to be susceptible to elevated temperature more than 3S °C such as Rhizobium le.,gpminosarufII bv.viceae (ARC 206 F and ARC 207 F) and Rhizobium legumino.,arumby. tYi/olii (ARC 103).5. Four rhizobia 1 strains tolerant to high temperature representing peanut Bradyrhizobium (3456 T 40°C and 619 T 45°C) andBradyrhizobium jqponicIlm (3407 T 40°C and 138 T 45°C) wereselected for further competence study.3- Effect of mineral nitrogen levels on survival of rhizobia:1. All tested strains were able to grow and survive under stress of200ppm N as KNO, (20 kg N I fed) in YEM medium 2. Strains representing Rhizobium le~",i'lO.<arum bv. viceae, bv. trifolii,bv. pha.<eoliand Bradyrhizobium sp. (Peanut) were able to tolerate upto 300ppm N111 liquid medium.3.A11 tested strains representing R. meliloti were able to tolerate up to400ppm N as KNO, (ARC 1, ARC 2, and ARC 3) . The other twostrains (ARC 6 and A 2) were superior in surviving up to 500 ppm N.4. With increasing the dose of mineral nitrogen up to

600 ppm N(KNO,), no growth was observed for any of the tested strains.5.Two rhizobial strains Bradyrhizobium sp. nodulating peanut(619 T300 ppm N .and 3456 T 500 ppm N) and two Bradyrhiz(lhi~;'ljaponiCllm strains (138 T 200 ppm Nand 3407 T 500 ppm N) wereselected for symbiotic perfonnance evaluation as compared tosensitive original ones.4- Effect of acidity and alkalini~y on survival of rhizobia: 1.The different rhizobial strains under investigation varied widely in their growth tolerance to pH values in YEM medium. The pH valuemost favourable for growth was 7.0 . A corresponding decrease ingrowth of all tested strains was recorded with the increase or decreasein pH values outside 7.0 .1. Strains of R !cguminosarum bv. phaseoli ARC 302 and peanutBradyrhizobium 618, 3339 and 601 were more tolerant to lower pHvalues up to 5. The growth rate ranged from 72-99 % as comparedwith growth at pH 7 .3. Regarding alkaline pH values tested only seven out of twenty - fiverhizobial strains were able to tolerate pH values up to .0 thesestrains were R. meliloti (ARC 103), R le.guminosarnm bv. phaseoli(UMR), peanut Bradyrhizobium (618) and .!3radyrhizohiumjaponicum (138 and 3407).4- Effect of desiccation on survival of rhizobia: 1. Growth and survival of representative five strains of R.leguminosarum by. viceae and peanut Bradyrhizobium as affected bydesiccation for 24 ..96 hr were investigated. Drying. stress wasimposed on the legume seeds coated with the rhizobial strains as peat_ based inoculant. kept in CaCh desiccator for 96 hr. Survival ofrhizobia was followed by determination of the viable rhizobial cellsnumber (log 10) per seed at intervals of 24 hr. 2. The growth rate of the rhizobial strains generally declined byincreasing the duration of desiccation. No strain from the two speciestested was able to survive desiccation up to 96 hr. However, one strain(ICARD A 441) of R. leguminosarum bv. »tceae and two strains (619and 3339) of Bradyrhizobium sp. retained viability after 72 hr. ofdrying stress. The .viability rate recorded was 42.9,52.3 and 50.1%respectively. 3. Comparing the viability responses as affected by desiccation after 2hr, R leguminosarum bv. viceae strains showed consistently lower growth rate than Bradyrhizobium sp. strains. The viability rate wasbetween 49.1 70.4% and between 93.5 - 98.7% for II.legum;nosarum bv. »tceae and Bradyrhizobium sp, respectively, ascompared to initial count After 48 hr of desiccation exposure, allstrains tested were able to tolerate drying except R. leguminosarumbv. viceae ARC 200F which showed no growth. 4. The obtained results show the importance of drying as factor affects the viability and survival of the rhizobial strains in free living state. However, comparison of survival under drying conditions indicatedthat Bradyrhizobium sp. (peanut) strains were more tolerant todesiccation than strains of Rhizobium tested over short periOds (24 hr):5. It appears that exposure to desiccation can be applied to select some rhizobia} strains (R. le.&JUminosarumby. vtceae, lCARDA 441) not seriously affected under stress of drying conditions.5~Symbiotic performance of stress tolerant rhizobia strains: ,In the present study , some rhizobial strains tolerant to differentsoil~stress factors were selected and their competence with' specific hostwere evaluated as well 5.1. 'Y'iWbiUPJ ""wniAAsslUm by. vil<eM toltranl to djt!eranl corgnlraiions gfNaCI with taba bean under soil salinity stress : 1- Inoculation of faba bean plants withR!Jizobium }egu.rn~nosarumbv.viceae ARC 200 F'and~CARDA 441 (tolerant to 1 and 40/0 NaClrespectively) resulted in significant increases in nodules number anddry weight, dry matter and nitrogen content under stress ofNaClsalinized soil up to 6% as compared to uninoculated plants. StrainIcARDA 441 was superior in the above mentioned parameterscompared to ARC 200F2- Strain ARC 200 F was able to induce few nodules (6 nodules I plant)on faba bean plants under stress of 0.80/0 NaCI salinized soil.However, strain ICARDA 441 was highly infective, the number ofnodules induced was 21 nodules / plant under the same level of soilsalinity.3- At high soil salinity (0.8% NaCI) which greatly depressed the growthof uninoculated plants (2.88 g1plant, 75 DAP) , there was bettergrowth of 4.02 g/'plant due to inoculation with ICARDA 441. Thesefindings indicated that Rhizobium lezu.minosarum. by. viceae cantolerate higher levels of soil salinity than the host plant.5.2. Bradvrhizobium sp. strains tolerant to high temperature with peanut: 1- Inoculation with Bradyrhizobium sp. strains (peanut) tolerant to hightemperature (40 and 45°C) increased the number of nodules/plant.up to 95 (3456 T 40 OC) and 106 (619 T 45°C) as compared withinoculated plants with original sensitive ones (3456 and 619) , thenumber of nodules formed ranged from 85 to 99 nodules/plant.2- The nodules formed on peanut plants inoculated with high temperaturetolerant strains were higher in dry weights (680-743 mg/plant).However,

Brad~rhizob;um peanut strain 3456 T 40°C was thesuperior among the four strains tested in producing higher weightnodules of 743 mg/plant. Peanut plants inoculated with originalstrains induced lower nodules dry weight (503-523 mg/plant).3- After 75 DAP, the influence of ill0culation with 3456 T 40°C and 619T 450C was promotive and produced the highest dry matter yield of19.9 and I 8.6 g/plant respectively, as compared to 10.1 and 1l.6 g Jplant for sensitive original strains.4- Inoculation with the high temperature tolerant strains seemed to be superior in increasing the nitrogen content of shoots by 82.7-I 89%(45 DAP) and 88.6-139.2% (75 DAP) over inoculated plants withsensitive original ones.5.3. Bradyrhizobiumja onicumstrains tolerant to hi h tem erature with so bean : 1- At 45 DAP no significant differences were obtained in nodulesnumber of soybean plants inoculated with original (3407 and 138)and their high temperature tolerant derivatives (3407 T 40°C and138 T 450C). where averages recorded were 105-109 and 99-11 5nodules/plant resp~ctively. The same trend was noticed with olderplants, 75 OAP.2- At the plant age of75 days, an increase of 53.3 and 47.6°Atin nodules dry weight were attributed to inoculation with 3407 T 40°C. and 138T 450C respectively over inoculation with strains 3407 and 138.3. The rate of increase in plant dry matter yield differed according torhizobial strain character as tolerant or sensitive to high remperaturPercentages of increase in plant dry biomass of 17.4 and 1.8 wererecorded for 3407 T 40·C and 138 T 45·C respectively.4- For nitrogen content a'n increase of 154.7% was recorded for soybeanplants inoculated with 3407 T 40 ·C over that inoculated withoriginal strain 3407 .5.4. Brad rtlizobium s. eanut strains tolerant to mineral nitro an under stresS of soil nitrogen1_ At 45 DAP irrespective of .the rhizobia! strain, nodules numberdecreased with increasing the level of nitrogen in the soil being lowerat 75 kg N/fed (7-27 nodules/plant) than at 20 kg N/fed (19-42noduels/plant). However, peanut plants inoculated with nitrogentolerant rhizobial strains 619 T 300 ppm Nand 3456 T 500 ppm Nformed higher number of nodules 20 and 27/plant respectively, undernitrogen fertilized soil with 75 kg N/fed compared to sensitiveoriginal strains (7 _ 10 nodules I plant). The corresponding valuesafter 75 DAP were 22-23 for the original strains and 27 - 31noduelslplant for their nitrogen tolerant derivatives ones.2- Nodules dry weight drastically decreased by application of higherdoses of nitrogen (75 kg N/fed) at the two periods of plant sampling.This finding was clear for inoculation with original Bradyrhizobiumpeanut ones. 3- Competence of rhizobia! strains tested tolerant to mineral nitrogen wasmore pronounced under stress of 50 kg N/fed than sensitive onesregarding increased' shoots dry weight and nitrogen content.1- At 75 DAP, the two Bra<Jyrhizobium japonicum selected strains tolerant to nitrogen showed different infectivity competence ascompared to the original sensitive ones. The higher infectivity of 94nodules / plant of 3407 T 500 ppm N was demonstrated at 75 kg N/fed than the original one (25 nodules /plant). However, littlefluctuation of no significancy in nwuber of nodules was recordedbetween strain 138 (104 nodules/plant) and strain 138 T 200 ppm N(114 nodules/plant) under stress of the same dose of nitrogen..2- The same trend was observed regarding uodll1es dry weight and dry,weight of plant shoots. In the majority of cases, inoculation withBradyrhizohium japonicum 3407 T 500 ppm resulted in higher dryweight of both nodll1es and shoots as compared to 3407 at the twoperiods of sampling. In contrast, there was no significant differencebetween 138 and T 200 ppm N regarding the above mentioned parameters.3- High significant quantities of nitrogen 781, 635 and 675 mg/plantwere accw,nulated in shoot tissues of soybean plants inoculated with3407 T 500 ppm N under stress of 20, 50 and 75 kg N/fedrespectively. In contrast, the influence of inoculation with 138 T 200ppm N on soybean shoots nitrogen content was nnpromotive (201,284 and 251 mg I plant) as.comparedto Bradyrhizobium jCJponicum138 original one (464 , 384 and 311 mg N/plant) .5.6. Bradyrhizobium sQ. (oeanutyand BradWizQtl1!ml.§QnicY...fJJ tolerantto low and high pH values1- One strain represent Bradyrhizobium sp. (peanut) was tolerant to lowpH 5 (618 T pH 5) and high pH 10 (618 T pH 10). Also, three strainsrepresent Bradl'yhizobium japonicum were loleranl.to high pH value(3407 T pH 11 and 138 T pH 11) and low pH 5 (3407 T pH 5) wereselected. The symbiotic ·performance of the obtained strains wereinvestigated.2- Uninoculated peanut and soybean plants were free of nodIlles. At 45 OAP, however, inoculation of peanut or soybean plants with originalor tolerant rhizobial strains resulted in induction of high number ofnodules/plant for peanut (25-44) and soybean (54-92) . Nodulesnumber/plant increased up to 51-99 for peanut and 105-174 forsoybean by increasing the plant age (75 DAP) .3- On the other hand, tolerance of the rhizobial strains tested

to high pH values of 10 and. 11 was parallel to increase the munber of nodulesinduced for the two intervals of plant sampling of 4S and 7S DAP.The corresponding numbers of nodules I plant recorded at 45 OAPwere 44~92 and 84 for 618 ,TpH 10,3407 T pH 11 and 138 T pH 11respectively. Ho~ever, -at 75 DAP the number of nodules/planlincreased to 91, 174 and 123 in the same order.4- The dry weight of nodules was found to have the same trend asnodulation, this fipding .was recorded for peanut and soybeanrhizobial strains tested.5.- Inoculation with alkaline (pH 10 and 11) tolerant Bradyrhizobium sp.(618 T pH 10yandBradyrhiznbium japonicum (3407 T pH 11 and138 T pH 11) were superior in increasing peanut and soybean plant'growth compared to original and acidity (pH 5) tolerant ones.6- It was clear that inoculated peanut plants with 618 T pH 10 was. ' superior in increasing nitrogen content (396 mg I plant) as comparedwith uninocu1ated (190 mglplant)~ inoculated with 618 T pH 5 (247mg/plant) and original strain 618 (225 mg / plant).7_ The nitrogen content of .soybean plants showed the same trend as , peanut plants. the tolerant p" japonieum strains tolerant to pH II(3407 T pH 11 and 138 T pH 11) were more effective inaccumulating more atmospheric nitrogen than the original ones.5.7. Rhizobium leauminosarum by. viceae strains tolerant to desiccation1- Two R. !eguminosarum bv viceae strains ARC 200 F and ICARD A441 were selected as low and high desiccation tolerant, respectively.The infectivity and effeciency of the two strains were evaluated withfaba bean under soil drought stress of75 ,60 ,45 and 30% WHC.2- Uninoculated faba bean plants formed a considerable number ofnodules being 45 and 7 nodules / plant at 75 and 30% soil WHCrespectively. Inoculated plants with desiccation tolerant strainsbeared higher number of nodules / plant being 114 and 13 (ARC200F) or 135 and 30 (ICARDA 441) at 75 and 300/0 WHC respecti vely.3- Generally, the highest nodules number anddry weight were inducedon faba bean plants inoculated with ICARDA 441 which recordedmore desiccation tolerance up to 72 hrs.4- The largest estimates of shoots dry weight were for inoculation withICARDA 441 being 9.7 and 8.18 g /plant at 45 and 30% WHCrespectively. The same trend was also observed for shoots nitrogencontent of95.1, 106.7 and 132.5 mg/plant for uninoculated , inoculatedwith ARC 200 F and inoculated plants with ICARDA 441 respectively at30%WHC.