Species Specific Rhizobium Inoculation on Seedling Growth of Albizia lebbeck and Acacia catechu Under Water Stress Conditions

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ABSTRACT

Background: Diverse group of rhizobia nodulate tree legumes. These nodulating bacteria have great potential with these tree legumes which are usually exposed to arid or water stress environmental conditions. Plant Growth Promoting Rhizobacteria (PGPR) can have positive effects on vigor and productivity, especially under stress conditions. Methods: The effect of water stress on four selected strains of Rhizobium species isolated from Albizia lebbeck, Delbergia sissoo, Acacia catechu and rhizospheric soils of legume crops were studied on A. lebbeck and A. catechu seedlings growth. The water stress (0, -3, -5, -8 and -10 bars) were obtained using Polyethylene glycol 6000 (PEG-6000) solutions. Results: A decrease in water potential produced a marked reduction in germination percentage, seedling germination and seed vigor index. Out of the four isolates tested, Rhizobium isolated from A. lebbeck and A. catechu performed better in respect of germination percentage, Seedling Vigor Index (SVI), root and shoot length, number of leaves, secondary root formation and total biomass yield, compared to other strains. Conclusion: Growth responses varied between the different strains, these differences in germination ability of A. lebbeck and A. catechu might be attributed to intraspecific interaction resulting from the effects of natural selection and selected strains could assist in the rehabilitation of agroforestry by overcoming the effect of water stress.

Key words: Rhizobium, A. lebbeck, A. catechu, water stress, germination

INTRODUCTION

Leguminous nitrogen fixing tree species can play a major role in improving productivity of degraded forest soils. Nitrogen fixing tree are important in afforestation of degraded lands and are widely grown in India. These stabilize sandy and eroded soil and exploit deep underground water by virtue of its extensive root system.

Of the different factors limiting the forest productivity, drought is the major important one, affecting the world food security and sustainability in agricultural production. The microbiotic, abiotic and biotic factors can all manipulate Rhizobium populations but it is the abiotic cause which is the most acknowledged and of these, drought is often quoted as one of the most imperative factors potentially limiting the Rhizobium-legume symbiosis. Rhizobia are affected by water stress in two ways, during drying and in any following rewetting phase. During drought, the thickness of water films around soil particles and the neck diameter of water filled pores decrease. As rhizobia are motile, this affects their movement and size reduction.

The legume-Rhizobium symbiosis in general is known to be more sensitive to environmental stress (especially drought) than the uninfected legume, suggesting that Rhizobium is the more sensitive of the two partners. In forest tree practices, seed inoculation with Rhizobium is normally not adopted in the areas where A. lebbeck or A. catechu and other leguminous tree species are growing luxuriantly due to good inoculation while in the wasteland area the microbial population is supposed to be very low or totally absent, the practice of artificial inoculation of Rhizobium from the strain of same species will give better results by boosting the rhizobial population of such area and to achieve healthy plant growth. A large number of studies have been carried out on the effects of water stress on the germination of forest tree species. However, in none of the studies was the
selection of A. lebbeck or A. catechu with Rhizobium species under different water potential conditions. Keeping the above in view, the present investigation was undertaken to study the effects of Rhizobium inoculation on A. lebbeck and A. catechu seedling germination under decreased water potential conditions imposed by PEG-6000.

MATERIALS AND METHODS

Isolation of test bacteria: Rhizobia were cultured from nodules from a minimum of five seedlings each of Albizia lebbeck, Acacia catechu, Delbergia sissoo by surface-sterilizing each nodule with 5% hypochlorite, crushing and smearing onto Yeast Extract Mannitol (YEM) agar medium and incubating the plates at 25°C for 3 days. The cultures were transported to Aberdeen on mannitol agar slopes and cultured again on mannitol agar plates. A single colony microscopically identified as rhizobial after staining was excised from each plate to establish pure cultures (tested by streak plating and replicate plating on mannitol agar). Stock cultures were made up from these isolates to provide the inocula for further studies. The four bacterial strains (fourth one isolated from the rhizosphere of leguminous crop soil) used in this study were selected on the basis of nitrogen fixation and phytohormone Indole Acetic Acid (IAA) production (Table 1).

Seed sterilization and inoculation: The healthy and uniform seeds of A. lebbeck and A. catechu were sterilized by exposing to 70% alcohol in a beaker for one minute and then dipping in 0.2% HgCl₂ solution for 50 sec, finally washed 5 times with sterile distilled water. The surface sterilized seeds were treated with different Rhizobium isolates (T1-Albizia isolate, T2-D albergia isolate, T3-Acacia isolate and T4-rhizosphere isolate), all the isolates were grown in YEM broth having count of 10⁶ cfu mL⁻¹.

Experimental setup: Polyethylene glycol 6000 (PEG), HiMedia, Mumbai, India has been used in this study because of its ability as nonpermeating solute to lower the external water potential without penetrating the cell wall. The PEG was dissolved in sterile distilled water to prepare osmoticum solution of -3.0, -5.0, -8.0 and -10.0 bars water potential as per the method of 9. Three replicates of 20 seeds of each cultivar were germinated in two rolled Whatman filter papers with 10 mL of respective test solutions. The papers were replaced every 2 days to prevent accumulation of salts9. In order to prevent evaporation, each rolled paper was put into a sealed plastic bag. Seeds were allowed to germinate at 20±1°C in the dark for 16 days. To determine the toxic effects of the PEG solutions on germination, non-germinated seeds in each treatment were transferred to distilled water and counted for an additional 3 days. Germination percentage was recorded every 24 h for 9 days. For interpretation of results, average mean data has been used in the present study. Seedling Vigor Index (SVI) was calculated by multiplying germination (%) and seedling length (mm). Each treatment was analyzed with at least three replicates and Standard Deviation (SD) was calculated using Microsoft excel program.

Statistical method: The standard deviation and mean in the tables have been calculated using Microsoft excel.

RESULTS

In this study, different Rhizobium isolates were tested with seeds of A. lebbeck and A. catechu under various water potential levels. The criterion for seed germination was taken as the emergence of 2 mm radicle at the time of observation. The germination percentage was found to decrease with increase in moisture stress levels in Albizia and Acacia species (Table 2). Transfer of non-germinated seeds from PEG solution to the distilled water resulted in 100% germination regardless of osmotic potential (data not shown), therefore, it showed that PEG was non toxic to seeds. The germination of seedlings of both the species were more with rhizobial isolates from same plant species i.e., A. lebbeck by T1 and A. catechu by T3 rhizobial isolate (Table 2). Rhizobium isolated from test species i.e., A. lebbeck T1 was found to be the more.

| Table 1: Nitrorgenase activity and IAA production by Rhizobium isolates used in present study |
|-----------------------------------------------|-------------|-------------|-------------|-------------|
| Rhizobial isolates | Nitrorgenase activity (nmol C₂H₄ from H₂ protein) | IAA production (µM) |
|------------------|-----------------|--------------|
| T1               | 65.4            | 73.3         |
| T2               | 67.6            | 67.4         |
| T3               | 59.5            | 79.2         |
| T4               | 47.9            | 65.7         |

| Table 2: Effect of rhizobial isolates and moisture stress levels on germination (%) of A. lebbeck and A. catechu |
|-----------------------------------------------|-------------|-------------|-------------|-------------|
| Rhizobial isolates | Stress levels (bars) | A. lebbeck (day) | A. catechu (day) |
|------------------|-----------------|---------------|---------------|
| T1               | -3              | 50.3±5.6      | 90.3±7.1      | 76.7±4.2    | 94.0±9.1    |
|                  | -5              | 42.6±5.1      | 87.0±6.5      | 71.2±6.4    | 90.0±3.7    |
|                  | -8              | 24.3±3.4      | 62.3±4.3      | 66.6±7.3    | 85.4±5.8    |
|                  | -10             | 18.7±3.0      | 55.4±4.7      | 62.4±3.5    | 82.2±7.3    |
| T2               | 3               | 44.6±4.2      | 88.3±3.3      | 80.2±8.3    | 96.6±8.2    |
|                  | 5               | 36.3±3.8      | 76.0±6.4      | 76.5±4.8    | 93.0±4.7    |
|                  | 8               | 20.3±3.4      | 62.3±4.2      | 70.0±6.2    | 90.5±6.9    |
|                  | -10             | 10.5±5.1      | 50.6±3.8      | 67.7±4.3    | 85.5±5.2    |
| T3               | 3               | 38.6±3.9      | 72.4±7.0      | 82.6±5.7    | 100.0±9.5   |
|                  | -5              | 30.3±3.1      | 60.5±6.8      | 79.3±4.2    | 97.7±7.1    |
|                  | -8              | 24.6±2.7      | 52.0±5.3      | 73.6±6.2    | 90.2±7.6    |
|                  | -10             | 10.0±2.0      | 38.4±4.9      | 70.2±4.0    | 90.5±6.7    |
| T4               | 3               | 30.0±3.8      | 63.3±6.8      | 73.0±6.9    | 90.2±6.7    |
|                  | -5              | 20.2±3.0      | 52.8±6.2      | 70.2±4.7    | 84.6±5.6    |
|                  | -8              | 16.6±2.4      | 45.0±3.8      | 68.4±5.6    | 82.2±6.4    |
|                  | -10             | 5.3±1.1       | 32.3±4.1      | 62.0±4.2    | 78.6±5.7    |
| Control          | 0               | 40.3±4.3      | 84.6±7.5      | 72.5±6.1    | 92.5±8.6    |
resistant in terms of overcoming the different stress levels and tolerated maximum moisture stress of -10 bars compared to control and followed by other treatments T2, T3 and T4 (Table 2). Similarly the A. catechu seeds showed maximum germination (%) at all moisture tension levels in presence of Rhizobial isolate T3 followed by T2, T1 and T3 (Table 2). The SVI and biomass yield was also influenced by species specific rhizobial isolates. Although, the inhibition effect on germination percentage of seed increased with increase in water potential, the inhibitory effect was overcome by inoculation of rhizobial isolates (Table 3). The root and shoot formation was also found to decrease with increasing water stress levels, although Rhizobium inoculation could bring about a increase in root and shoot formation in water stressed A. lebbeck and A. catechu seedling over control seedlings where no stress was exerted. Rhizobium from the test species of A. catechu (T3) exhibited resistance with different stress levels and similar results were obtained with A. lebbeck (Table 4). The number of leaves and secondary root formation in A. lebbeck were also found to decrease with increase in moisture stress levels. Inoculation of rhizobial strains were able to form leaf emergence maximum to -5 bars and no growth was observed at -8 and -10 bars. The secondary root formation was observed up to -8 bars in T1 and T2 treatments (Table 5). In A. catechu no leaf formation was observed till 10th day of experiment. Isolates T1 and T3 and control showed leaf formation on 13th day, while in T2 no leaf formation observed till 16th day of study. Secondary roots formation was observed at -3 and -5 bars in T1 and T2, -3, -5 and -8 bars in T3 while only at -3 bars in T4 isolates (Table 5, 6).

Table 3: Effect of rhizobial isolates and moisture stress levels on Seedling Vigor Index (SVI) and biological yield of A. lebbeck and A. catechu

| Rhizobial isolates | Stress levels (bars) | SVI  | Biomass yield 14th day | SVI  | Biomass yield 14th day |
|--------------------|----------------------|------|------------------------|------|------------------------|
| T1                 | -3                   | 112.9±18.2 | 0.86±0.03 | 104.36±14.4 | 0.39±0.04 |
|                    | -5                   | 96.0±14.5  | 0.70±0.04 | 84.56±12.6  | 0.28±0.06 |
|                    | -8                   | 67.4±9.30  | 0.59±0.08 | 68.9±14.5   | 0.20±0.04 |
|                    | -10                  | 28.1±3.70  | 0.44±0.02 | 57.03±9.50  | 0.12±0.01 |
| T2                 | -3                   | 103.8±9.80 | 0.72±0.04 | 99.8±10.3   | 0.46±0.06 |
|                    | -5                   | 77.5±10.5  | 0.58±0.09 | 82.08±8.40  | 0.36±0.04 |
|                    | -8                   | 46.8±7.60  | 0.50±0.03 | 65.7±12.3   | 0.26±0.05 |
|                    | -10                  | 34.1±5.90  | 0.41±0.03 | 58.0±9.50   | 0.15±0.02 |
| T3                 | -3                   | 78.9±8.10  | 0.64±0.02 | 118.49±19.4 | 0.57±0.06 |
|                    | -5                   | 63.1±11.5  | 0.51±0.06 | 93.7±14.2   | 0.42±0.04 |
|                    | -8                   | 34.8±5.80  | 0.44±0.05 | 77.8±6.00   | 0.33±0.07 |
|                    | -10                  | 19.5±4.70  | 0.35±0.07 | 65.9±6.20   | 0.22±0.03 |
| T4                 | -3                   | 67.9±10.1  | 0.52±0.06 | 71.29±7.90  | 0.24±0.02 |
|                    | -5                   | 41.6±6.40  | 0.40±0.03 | 61.9±6.80   | 0.16±0.01 |
|                    | -8                   | 27.0±5.30  | 0.22±0.02 | 51.1±4.60   | 0.10±0.03 |
|                    | -10                  | 19.0±4.10  | 0.17±0.05 | 43.2±6.20   | 0.06±0.01 |
| Control            | 0                    | 95.94±8.7 | 0.60±0.07 | 82.06±11.4  | 0.38±0.04 |

Table 4: Effect of rhizobial isolates and moisture stress levels on root and shoot length of A. lebbeck and A. catechu

| Rhizobial isolates | Stress levels (bars) | 12th day root | 12th day shoot | 12th day root | 12th day shoot |
|--------------------|----------------------|---------------|---------------|---------------|---------------|
| T1                 | -3                   | 2.66±0.72     | 5.90±1.02     | 1.92±0.39     | 3.05±0.82     |
|                    | -5                   | 2.24±0.45     | 5.58±0.98     | 1.76±0.65     | 2.97±0.52     |
|                    | -8                   | 1.75±0.38     | 4.90±0.54     | 1.61±0.25     | 2.40±0.61     |
|                    | -10                  | 1.51±0.25     | 4.26±0.51     | 1.39±0.20     | 2.13±0.37     |
| T2                 | -3                   | 2.02±0.36     | 5.22±0.89     | 1.79±0.42     | 2.84±0.68     |
|                    | -5                   | 1.62±0.03     | 4.90±0.53     | 1.67±0.40     | 2.66±0.57     |
|                    | -8                   | 1.67±0.28     | 4.43±0.41     | 1.51±0.72     | 2.30±0.69     |
|                    | -10                  | 1.49±0.24     | 3.62±0.29     | 1.22±0.25     | 2.03±0.71     |
| T3                 | -3                   | 1.91±0.31     | 4.77±0.99     | 2.02±0.36     | 3.29±0.51     |
|                    | -5                   | 1.77±0.24     | 4.02±0.58     | 1.81±0.89     | 3.01±0.82     |
|                    | -8                   | 1.40±0.21     | 3.97±0.39     | 1.64±0.25     | 2.80±0.49     |
|                    | -10                  | 1.29±0.18     | 2.81±0.34     | 1.40±0.30     | 2.53±0.67     |
| T4                 | -3                   | 1.78±0.39     | 3.74±0.39     | 1.52±0.19     | 2.61±0.91     |
|                    | -5                   | 1.60±0.28     | 3.02±0.32     | 1.34±0.75     | 2.37±0.65     |
|                    | -8                   | 1.31±0.21     | 2.66±0.21     | 1.21±0.31     | 1.96±0.39     |
|                    | -10                  | 1.27±0.13     | 2.48±0.18     | 1.11±0.26     | 1.72±0.76     |
| Control            | 0                    | 2.00±0.72     | 5.50±1.04     | 1.89±0.87     | 2.90±0.62     |
Table 5: Effect of rhizobial isolates and moisture stress levels on leaf and secondary root formation of A. lebbeck

| Rhizobial isolates | Stress levels (bars) | Leaf formation (N.o.) | Secondary roots formation (N.o.) |
|-------------------|----------------------|------------------------|---------------------------------|
|                   | 7th day | 10th day | 13th day | 16th day | 7th day | 10th day | 13th day | 16th day |
| T1                | 7       | 5        | -4       | -10      | 1.0±0.10 | 1.30±0.23 | 2.30±0.89 | 2.30±0.87 | 12.1±2.30 | 19.6±5.20 | 26.7±7.20 | 29.3±7.10 |
|                   | -3       | -5       | -8       | -10      | 5.20±1.10 | 11.4±6.50 | 15.4±4.80 | 16.5±4.70 | 0.24±0.84 | 0.26±0.54 |
| T2                | -3       | -5       | -8       | -10      | 1.00±0.11 | 1.00±0.96 | 1.30±0.72 | 0.70±0.50 | 0.24±0.84 | 0.26±0.54 |
|                   | -3      | -5       | -8       | -10      | 4.70±1.00 | 09.7±3.80 | 14.3±7.10 | 18.1±5.30 | 02.6±0.54 |
| T3                | -3       | -5       | -8       | -10      | 0.66±0.15 | 1.00±0.12 | 1.0±0.11 | 8.30±1.00 | 11.3±2.70 | 15.1±3.50 | 15.1±3.60 |
|                   | -5       | -8       | -10      | -10      | 2.50±0.95 | 08.4±3.00 | 12.3±2.70 | 13.2±2.80 | -         |
| T4                | -3       | -5       | -8       | -10      | 0.30±0.09 | 0.66±0.10 | 0.66±0.16 | 5.70±1.40 | 10.6±3.50 | 13.4±3.90 | 13.4±4.10 |
|                   | -5       | -8       | -10      | -10      | 0.33±0.57 | 0.33±0.05 | 5.6±1.80 | 9.20±2.20 | 11.0±2.70 | 13.4±2.70 |
| Control           | -0       | -        | -        | -        | 0.66±0.07 | 1.00±0.11 | 1.30±0.19 | 10.1±3.50 | 16.6±4.10 | 24.1±4.20 | 26.1±7.80 |

-: No growth

Table 6: Effect of rhizobial isolates and moisture stress levels on leaf and secondary root formation of A. catechu

| Rhizobial isolates | Stress levels (bars) | Leaf formation (N.o.) | Secondary roots formation (N.o.) |
|-------------------|----------------------|------------------------|---------------------------------|
|                   | 7th day | 10th day | 13th day | 16th day | 7th day | 10th day | 13th day | 16th day |
| T1                | -3       | -5       | -8       | -10      | 0.33±0.10 | 0.33±0.09 | 4.1±0.87 | 6.30±1.60 | 8.20±3.40 | 9.20±2.50 |
|                   | -5       | -8       | -10      | -10      | 1.50±0.43 | 3.40±1.00 | 3.50±0.72 | -         | -         |
| T2                | -3       | -5       | -8       | -10      | 7.3±2.10 | 9.40±2.80 | 10.3±2.80 | 10.4±2.60 |
|                   | -5       | -8       | -10      | -10      | 3.4±1.00 | 5.30±1.60 | 7.50±2.60 | 8.10±1.30 |
| T3                | -3       | -5       | -8       | -10      | 1.60±0.69 | 2.00±0.08 | 8.3±2.80 | 11.7±3.10 | 12.5±3.10 | 16.3±3.90 |
|                   | -5       | -8       | -10      | -10      | 0.33±0.57 | 0.33±0.05 | 5.6±1.80 | 9.20±2.20 | 11.0±2.70 | 13.4±2.70 |
| T4                | -3       | -5       | -8       | -10      | 1.60±0.69 | 2.00±0.08 | 8.3±2.80 | 11.7±3.10 | 12.5±3.10 | 16.3±3.90 |
|                   | -5       | -8       | -10      | -10      | 5.1±1.20 | 8.10±2.70 | 12.3±3.40 | 15.6±4.10 |
| Control           | -0       | -        | -        | -        | 0.30±0.04 | 0.66±0.05 | 5.1±1.20 | 8.10±2.70 | 12.3±3.40 | 15.6±4.10 |

-: No growth

**DISCUSSION**

Germination studies of ecologically important nitrogen fixing trees need priority attention to include them in plantation programs. Seed dormancy is an important constraint faced in the majority of hard coat species. Thus, enhancing seed germination by treatment seeds is an important aspect. The time required for effective treatment differs between species and related with seed coat thickness. In the present study, different rhizobial isolates were tested under various stress levels for their effectiveness in root colonization and promoting plant growth parameters. The retardation or suppression of various seedlings growth was observed proportionally to the increasing moisture stress levels (Table 2-6). This can be due to the retardation of mobilization of reserves of carbohydrates and proteins which are easily assessable when there is no moisture stress. Rhizobium isolated from test species i.e., A. lebbeck (T1) and A. catechu (T3) were found to be resistant against various water potential compared to other (T2 and T4) rhizobial species.

The various physiological response of plant to water scarcity varies with the duration of stress. Large number of processes is altered even by a very mild stress. It was observed that artificial inoculation of Rhizobium from the strain of same species gave better results in most of the
parameters to overcome water stress levels. The seeds which were treated with Rhizobium were able to tolerate the stress or duration of stress to a greater extent than control and other treatments (Table 3, 4). Any treatment that is used to overcome physical seed dormancy is designed mainly to soften, puncture, wear away or split the seed coat in order to render it permeable without damaging the embryo and endosperm within it\textsuperscript{11}. It is quite possible that Rhizobium treatment has softened the seed coat and made it permeable. Reduced germination under water stress conditions may be attributed to the effect that seeds seemingly develop an osmotically enforced “dormancy” under water stress conditions which may be an adaptive strategy of seeds to prevent germination under stressful environment thus ensuring proper establishment of the seedlings\textsuperscript{12,13}.

Osmotic stress caused a significant reduction in seedling germination and other plant growth parameters, indicating that seedlings were under stress. Similar observations under stress conditions were made by\textsuperscript{14} in Sorghum spp., wheat\textsuperscript{15}, Chenopodium spp.\textsuperscript{16} The germinability of seeds decreased with increasing the level of water stress, not only the germination was inhibited but the extension growth of the seedlings was also obstructed. These results show that radicle and plumule growth of the seedlings was greatly adversely affected by water stress. Slow and poor germination under water stress is obviously due to decreased water potential of the germination medium which restricts the water availability to the seeds\textsuperscript{17-19}. As water moisture is one of the primary requirement in seed germination the water stress developed by PEG reduced germination greatly in this study.

The application of selected rhizobial strains resulted in adverting the effect of moisture stress, these positive effects of bacteria on seed germination might be attributed to increased water use efficiency, stimulation of root growth by production of phytohormones and/or softening of seed coat by enzymatic activities and lowering of plant ethylene concentrations. Bacterial IAA production under water stressed conditions may explain their effectiveness in promoting plant growth and shoot water content increasing plant drought tolerance\textsuperscript{20,21}. The differences in these results may also be due to the adaptation of tree legumes when inoculated with their specific rhizobia. The non-species specific rhizobia were not able to make interaction with selected tree legume seeds, thus resulted in less tolerance of increasing water stress levels. Although the rhizobial strains T2 and T4 have nitrogen fixing and IAA producing capabilities, yet they were not able to tolerate the increasing water potential levels compared to species specific strains T1 and T3. The selected Rhizobium strains obtained in this study are excellent models to study the precise mechanism(s) of such interaction of adoption and to elucidate the role of genetics of drought tolerance.

**CONCLUSION**

The drought tolerant pattern found among the indigenous rhizobial strains are reflecting the environmental stresses pressure predominant in their locations and are very good examples of the importance of using efficient-indigenous rhizobial strain for plant inoculation in each specific area. Rhizobium with the genetic potential for increased tolerance to drought and/or salinity could enhance production of food and forage in legume in semiarid and arid regions of the world.

**REFERENCES**

1. Rasanen, L.A. and K. Lindstrom, 2003. Effects of biotic and abiotic constraints on the symbiosis between rhizobia and the tropical leguminous trees Acacia and Prosopis. Indian J. Exp. Biol., 41: 1142-1159.
2. Abdelmoumen, H., A. Filali-Maltouf, M. N eyra, A. Belabed and M.M. El Idrissi, 1999. Effect of high salts concentrations on the growth of rhizobia and responses to added osmotica. J. Applied Microbiol., 86: 889-898.
3. Serraj, R., T.R. Sinclair and L.C. Purcell, 1999. Symbiotic N₂ fixation response to drought. J. Exp. Bot., 50: 143-155.
4. Swaine, E., M. Swaine and K. Killham, 2007. Effects of drought on isolates of Bradyrhizobium elkanii cultured from Albizia adianthifolia seedlings of different provenances. Agroforestry Syst., 69: 135-145.
5. Yang, J., J.W. Kloepper and C.M. Ryu, 2009. Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci., 14: 1-4.
6. Jamaluddin, V.S. Dadwal and V.S. Chouhan, 1995. Efficacy of different Rhizobium strains of forest trees species on Albizia lebbeck. Indian Forester, 121: 647-650.
7. Rincon, A., F. Valladares, T.E. Gimeno and J.J. Pueyo, 2008. Water stress responses of two Mediterranean tree species influenced by native soil microorganisms and inoculation with a plant growth promoting rhizobacterium. Tree Physiol., 28: 1693-1701.
8. Rehman S., P.J.C. Harris, W.W. Boren and J. Wilkins, 1996. The effects of sodium chloride on germination and the potassium and calcium contents of Acacia seeds. Seed Sci. Technol., 25: 45-57.
9. Mayak, S., T. Tirosh and B.R. Glick, 2004. Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci., 166: 525-530.

10. Marulanda, A., J.M. Barea and R. Azcon, 2009. Stimulation of plant growth and drought tolerance by native microorganisms (AM fungi and bacteria) from dry environments. Mechanisms related to bacterial effectiveness. J. Plant Growth Regul., 28: 115-124.

11. Mayak, S., T. Tirosh and B.R. Glick, 2004. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol. Biochem., 42: 565-572.

12. Hardikar, S.A. and A.N. Pandey, 2008. Growth, water status and nutrient accumulation of seedlings of Acacia Senegal (L.) Willd. in response to soil salinity. Anales de Biologia, 30: 17-28.

13. Tilki, F. and H. Dirik, 2007. Seed germination of three provenances of Pinus brutia (Ten.) as influenced by stratification, temperature and water stress. J. Environ. Biol., 28: 133-136.

14. Gill, P.K., A.D. Sharma, P. Singh and S.S. Bhullar, 2001. Effect of various abiotic stresses on the growth, soluble sugars and water relations of sorghum seedlings grown in light and darkness. Bulg. J. Plant Physiol., 27: 72-84.

15. Ceccon, E., A.A. Rogel, A.M. Romero and I. Toledo, 2011. The effect of inoculation of an indigenous bacteria on the early growth of Acacia farnesiana in a degraded area. Cerne, 18: 49-57.

16. Shaikh, N.R and M.B. Gandhi, 2009. Rhizobia from saline-environment nodulating Vigna mungo (L) and its significance in agricultural biotechnology. J. Environ. Res. Dev., 3: 1154-1163.

17. Soltani, A., S. Galeshi, E. Zeinali and N. Latifi, 2002. Germination, seed reserve utilization and seedling growth of chickpea as affected by salinity and seed size. Seed Sci. Technol., 30: 51-60.

18. Dimkpa, C., T. Weinand and F. Asch, 2009. Plant-rhizobacteria interactions alleviate abiotic stress conditions. Plant Cell. Environ., 32: 1682-1694.

19. Zaharan, H.H., 2010. Legumes-Microbes Interactions Under Stressed Environments. In: Microbes for Legume Improvement, Khan, M.S., A. Zaidi and J. Musarrat (Eds.). Springer-Verlag, USA., pp: 353-387.

20. Aldesuquy, H.S., Z.A. Baka and B.M. Mickky, 2013. Wheat can acclimate to seawater by pretreatment with kinetin and spermine through osmotic adjustment and solutes allocation. J. Stress Physiol. Biochem., 9: 181-198.