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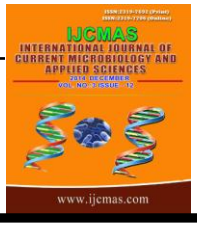

Original Research Article

Symbiotic effectivity of high temperature tolerant mungbean (*Vigna radiata*) rhizobia under different temperature conditions

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Keywords

Mungbean, Temperature tolerance, Rhizobia, Extracellular polysaccharide, Trehalose, Symbiosis, Nodulation

A B S T R A C T

Ninety one rhizobial isolates were obtained from the nodules of mungbean (*Vigna radiata*) crop grown in different districts of Haryana. Authenticated rhizobia by plant infection test were used to select thermo tolerant rhizobia. Rhizobial isolates showed luxuriant growth at temperature ranging from 30-40ºC, while at 45ºC only 2 isolates, MR23 and MS57 could grow. These two thermo tolerant isolates MR23 and MS57 produced less extra cellular polysaccharides and accumulated more intracellular trehalose at higher temperature. New polypeptide bands of approximately 75 and 21 kDa were observed in the rhizobial isolate MR23 and of approximately 20 kDa in the rhizobial isolate MS57 when grown at 45ºC in comparison to growth at normal temperature. Symbiotic effectiveness of high temperature tolerant MR23 and MS57 and temperature sensitive MR14 rhizobial isolate along with reference strain MB703, was evaluated under sterilized conditions in chillum jars with temperature regime of 31 to 40ºC; leonard jars with temperature regime of 37 to 49ºC and under un sterilized pots conditions with temperature regime of 31.7 to 41ºC. Beneficial effect of high temperature tolerant rhizobial isolates MR23 and MS57 inoculation was more pronounced at higher temperature regime under leonard jar conditions.

Introduction

Mungbean (*Vigna radiata*) is one of the important legumes and a well-known economic crop in tropical and subtropical countries. Mungbean is grown during spring, summer and kharif season in Northern India, while grown during Rabi season in Southern India. As a leguminous plant, mungbean is nodulated by rhizobia, causing the formation of nodules and establishing a nitrogen-fixing symbiosis (Dudeja et al., 2012).

Abiotic stresses severely affect the growth, nodulation and yield of mungbean (*Vigna radiata*). In mungbean, nearly 40-100% yield losses are due to various environmental stresses depending upon the geographical region. Among the environmental stresses limiting legume-*Rhizobium* associations, temperature is the most influential especially in systems where tropical legumes are introduced into temperate regions (Sheokand et al., 2012).

Temperature affects the legume-*Rhizobium* symbiosis either directly, by limiting the growth of the micro symbiont and/or indirectly, by regulating the growth of the macrosymbiont (Munevar and Wollum, 1981; Dudeja and Khurana, 1994). Even before nodule establishment, root zone temperature influences the rhizobial survival in soil as well as the exchange of molecular signals between the symbiotic partners (Dudeja and Khurana, 1988a, b, 1989a; Dudeja et al., 1993; Raghuwanshi et al., 1994; Duhan and Dudeja, 1998). High temperature was found to have an inhibitory effect on adherence of bacteria to root hair; root hair formation and infection thread formation (Dudeja and Khurana, 1989b).

The tolerance of rhizobia to high temperature *in vitro* does not always correlate with the phenotype during symbiosis under the same conditions, at least for bradyrhizobia nodulating soybean and *Rhizobium phaseoli* nodulating common bean (Zahran, 1999). However, in other studies, strain tolerance to high temperature assessed *in vitro* correlated well with the results obtained in symbiotic effectivity (Kulkarni and Nautiyal, 1999). High temperature tolerant strains (60ºC), NBRI12, NBRI329, NBRI330 and NBRI332 were able to nodulate *Prosopis juliflora* growing in glasshouse and plants had higher shoot dry weight in comparison to un inoculated plants growing in nursery. Rhizobial strains isolated from the leguminous trees, *Gliricidia, Lonchocarpus* and *Leucaena* were capable of fixing nitrogen with bean at temperature of 40ºC (Hungria et al., 1993). Effect of elevated temperature on nitrogen fixation of nodulated alfalfa plants showed that plant and nodule dry weight severely affected by the high temperature (Aranjuelo et al., 2007). Greenhouse experiment carried out at 28 and 38ºC to study the nitrogen fixing capacity of the soybean isolates showed that ten isolates had a symbiotic index of 80% effectiveness or greater compared to nitrogen fertilized treatments at 28ºC (Rahmani et al., 2009). Some thermo tolerant isolates showed good nitrogen fixing performance at 38ºC. Temperature tolerant pigeon pea rhizobia did not show any differences in their effectivity under greenhouse experiment (Gopalakrishnan and Dudeja, 1999). Other attempts made to improve nodulation and competitiveness of pigeonpea and mungbean could not succeed (Dudeja and Khurana, 1988c; Chaudhary et al., 1999). Since there are wide variations in temperature from -2 to 47^0C in Northern India and under such adverse conditions there is need to have temperature tolerant rhizobia as other rhizobia showed very poor survival under field conditions (Dudeja and Khurana, 1989a). There fore in the present investigation temperature tolerant rhizobia were isolated from mungbean and these were evaluated under different temperature regimes under green house conditions.

Materials and Methods

Isolation of mungbean rhizobia

Root nodules samples of mungbean (*Vigna radiata*) were collected from different districts of Haryana (Sirsa, Hisar and Rewari) and were washed gently with tap water to remove the soil. Nodules were separated, placed in petri-plates and surface sterilized by dipping in 0.1% $HgCl_2$ for 2 min, followed by 70% ethanol and 5 times washing with sterilized distilled water (Vincent, 1970). Crushed nodules were streaked on yeast extract mannitol agar (YEMA) medium plates and incubated at 28 ± 2^0C. Single colonies were re streaked on YEMA medium plates and 91 pure cultures were obtained and were stored at 4 ± 1^0C on slants for further studies.

Authentication of rhizobia by plant infectivity test

All the 91 rhizobial isolates were authenticated by plant infection test using mungbean seeds (cv MH 421) under sterilized conditions in coffee cups (Giri and Dudeja, 2013). Seeds were surface-sterilized with a 0.2% $HgCl_2$ followed by 70% ethanol and finally rinsed in five changes of sterile water. Sterilized seeds were inoculated with log phase growing rhizobial cultures (10^4 – 10^5 cfu/seed) and sown in sterilized coffee cups containing sand in triplicate. Seedlings were watered with sterilized tap water. Nodule formation was scored after 50 days.

Selection of thermo tolerant mungbean rhizobia

Freshly grown cultures of rhizobial isolates were spotted on YEMA plates and the plates were incubated at different temperatures of 30, 35, 40 and 45ºC to screen for their maximum growth temperature. After 3 days of incubation, rhizobial growth was recorded by visual observation compared to control treatments incubated at 30ºC. Further all rhizobial isolates were also screened in YEM broth. Rhizobial suspensions of all isolates were inoculated into YEM broth tubes and these tubes were incubated at different temperatures. After 5 days of incubation, absorbance of the resultant growth was measured using a spectrophotometer at 600 nm.

Extracellular polysaccharide (EPS) production by thermo tolerant rhizobia

Freshly grown selected thermo tolerant rhizobial isolates were inoculated into 50 ml of YEM broth and incubated at 30^0C for 5 days. Cells were removed by centrifugation at 10,000xg for 10 min. Culture filtrate was mixed with three volumes of ethanol and after standing at 4^0C for 24 h, it was centrifuged. Weight of the precipitated EPS was measured after drying at 45^0C for 12 h.

Intracellular trehalose contents of thermo tolerant rhizobia

Log phase growing rhizobial cells from 5 ml broth were harvested and washed three times with ice cold distilled water by centrifugation. Ice cold 4.0 ml 0.5 M tricholoroacetic acid was added to rhizobial pellet. The mixture was incubated in refrigerator for 20 min with frequent shaking. The sample was centrifuged and the supernatant was collected. The trehalose contents in the supernatant were assayed by Anthrone reaction (Chi and Zhang, 2001).

Protein profile of thermo tolerant rhizobia

The protein profile of selected thermo tolerant rhizobial isolates was assayed by SDS-PAGE. Rhizobia were grown in YEM broth for 3-4 days. Cells collected by centrifugation and washed thrice with 0.1% saline Milli Q water. Samples containing 10 mg protein/ml were prepared and electrophoresis was carried out according to the method of Laemmli (1970). The stacking gel was 4% (w/v) acrylamide and resolving SDS-containing gel was 12.5% (w/v) acrylamide and was stained with Coomassie Brilliant Blue dye.

Efficacy of thermo tolerant rhizobial isolates in mungbean under greenhouse

Efficacy of thermo tolerant and thermo sensitive isolates was assessed under sterilized conditions in chillum jars and traditional Leonard jars, which are known to have 7 to 8^0C difference in root temperature (Dahiya and Khurana, 1981; Khurana and

Dudeja, 1981). Efficacy was further assessed under unsterilized conditions in pots. In each pot, 8 kg of sandy loam soil was added having pH 8.1; EC 0.34 dS m^{-1}; OC (%) 0.22; N (%) 0.028 and total P 17 Kg/ha. Under all these three conditions sowing was done using cv MH421 and were inoculated with different thermo tolerant rhizobia.

Experiments were conducted during the April month and soil temperature during the cropping period was monitored in chillums jar; leonard jars and in pots at 10cm depth using thermometers with soil probe. For plant growth and nodulation studies, the plants were uprooted after 50 days of emergence. Nodule number, nodule fresh weight, shoot height and plant biomass (root and shoot biomass) were recorded. The oven dried shoots were ground for the analysis of nitrogen content by Kjeldahl's method (Bremner, 1960).

Result and Discussion

Isolation and authentication of mungbean rhizobia

A total of 91 rhizobial isolates were obtained from the nodules of mungbean (*Vigna radiata*) crop grown in different parts of Haryana. Authenticity of rhizobia was done by plant infection test using mungbean as test host under sterilized conditions. Amongest these, 41 isolates were from Sirsa, 24 from Hisar and 26 from Rewari district. Nomenclature of the isolates was carried out representing their parent plant (first letter), region of origin (second letter) and isolate number (numeric figure) (Table 1).

Selection of thermo tolerant mungbean rhizobia

In vitro studies showed pronounced effect of high temperature on the rhizobial growth. In general, majority of the rhizobial isolates exhibited luxuriant growth at temperature ranging between 30-40ºC (Table 1). All isolates could grow on YEMA medium plates as well as in YEM broth incubated at 30 and 35ºC. At 40ºC, 77 isolates showed good growth while remaining showed no growth. Further increase in temperature led to noticeable decline in growth and at 45ºC, only 2 isolates, MR23 and MS57 could grow on YEMA medium plates as well in YEM broth. These two isolates were used for further studies

Extracellular polysaccharide (EPS) production and intracellular trehalose contents of thermo tolerant rhizobia

Thermo tolerant rhizobial isolates were characterized both under temperature stress and non stress conditions for their EPS production ability and trehalose contents. High temperature stress affected EPS production ability of high temperature tolerant rhizobial isolates (Fig. 1). Decrease in EPS production was observed by the rhizobial isolates in response to high temperature stress (45ºC). EPS production was reduced to 81.34% with rhizobial isolate MR23 and 76.19% with the rhizobial isolate MS57 under the influence of high temperature stress (45ºC) as compared to EPS production at normal temperature.

Since under temperature stress, trehalose is accumulated inside the cells. Therefore in thermo tolerant mungbean rhizobia, trehalose content in the presence and absence of stress conditions was determined. Under high temperature stress conditions (45ºC) increase in trehalose content was to the extent of 71.43% with rhizobial isolate MS57 and 26.53% with MR23 than as compared to non stress conditions (Fig. 1).

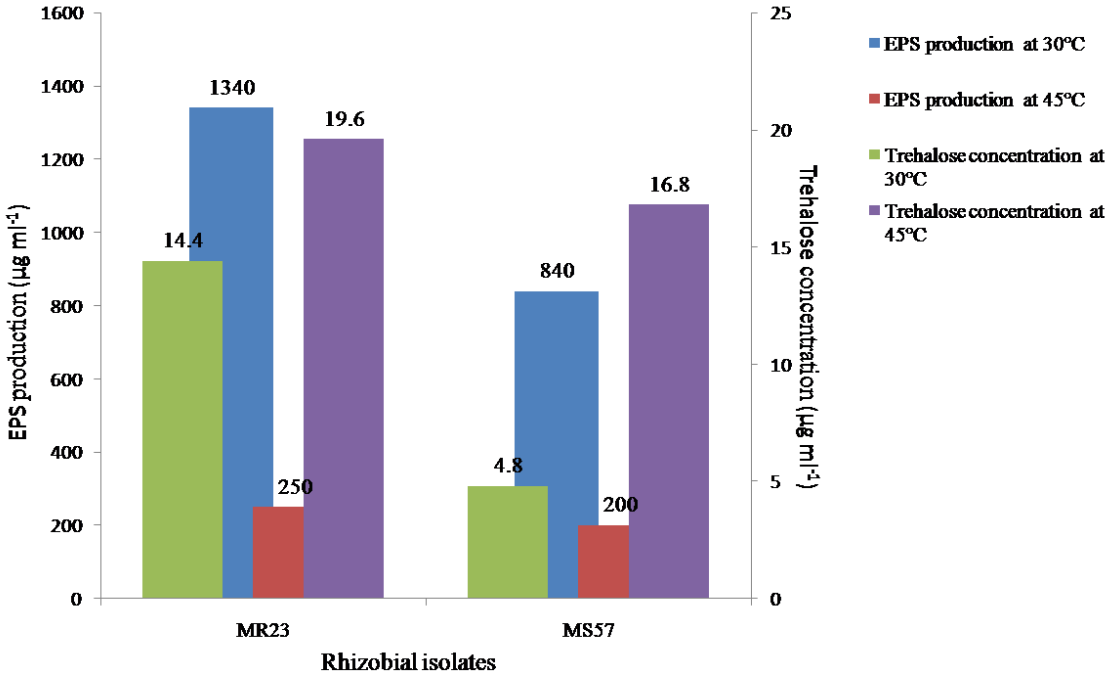

Fig.1 Effect of high temperature on EPS production and trehalose contents of thermo tolerant mungbean rhizobia

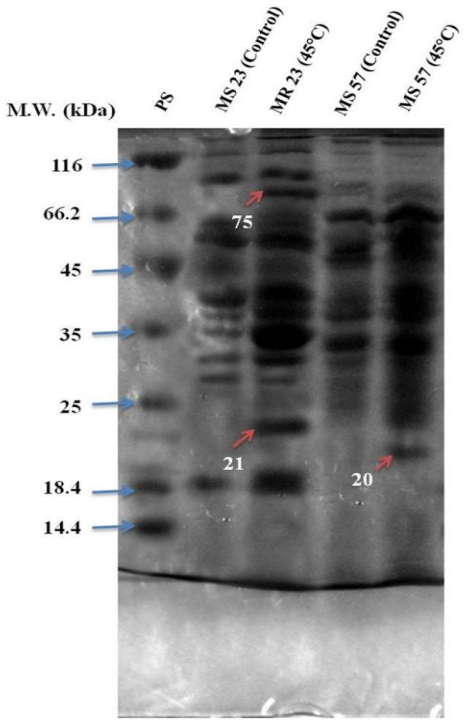

Fig.2 Effect of high temperature on whole cell protein patterns of thermo tolerant mungbean rhizobial isolates in 12.5% SDS-PAGE

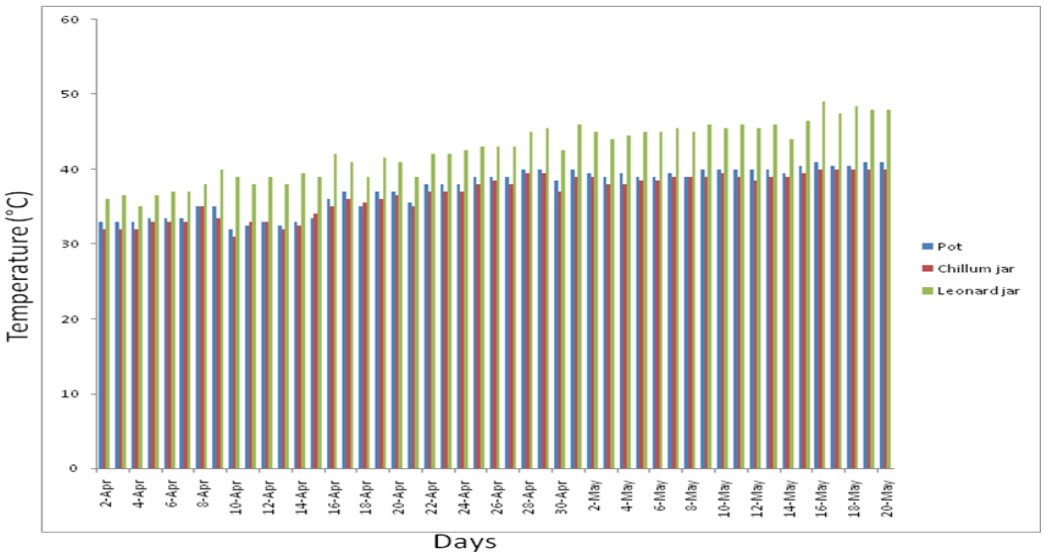

Fig.3 Soil temperature during the cropping season

Fig.4 Efficacy of thermo tolerant mungbean rhizobia in chillum and leonard jars

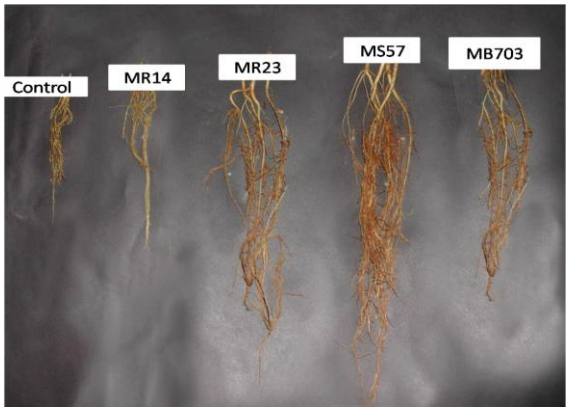

Fig.5 Efficacy of thermo tolerant mungbean rhizobia on root growth of mungbean in leonard jars

Table.1 Isolation and temperature tolerance of rhizobial isolates from mungbean plants collected from different districts of Haryana state

Location	Isolates showing growth at different temperatures		
	30 and 35ºC	40ºC	45 ºC
Sirsa	MS11, MS12, MS13, MS14, MS15, MS16, MS17, MS18, MS21, MS22, MS24, MS25, MS26, MS27, MS28, MS31, MS32, MS34, MS35, MS36, MS37, MS45, MS46, MS57, MS58, MS61, MS62, MS63, MS65, MS66, MS68, MS74, MS75, MS76, MS78, MS81, MS83, MS84, MS85, MS86, MS88	All the 41 isolates showed growth	Only MS57 showed growth
Hisar	MH11, MH13, MH14, MH16, MH17, MH18, MH31, MH41, MH64, MH65, MH71, MH72, MH73, MH74, MH75, MH76, MH77, MH78, MH81, MH82, MH83, MH84, MH87, MH88	Except isolates MH12, MH15, all other isolates showed growth	No isolate showed growth
Rewari	MR11, MR12, MR14, MR21, MR22, MR23, MR24, MR31, MR32, MR33, MR34, MR41, MR42, MR43, MR44, MR45, MR52, MR53, MR54, MR61, MR62, MR63, MR64, MR71, MR73, MR74	MR11, MR12, MR23, MR31, MR32, MR33, MR41, MR42, MR43, MR45, MR53, MR71, MR73, MR74 isolates showed growth	Only MR23 showed growth

Table.2 Efficacy of thermo tolerant rhizobial isolates in terms of nodulation and growth of mungbean under sterilized conditions of chillum and leonard jars

S. No.	**Treatments**		**Nodule (number plant^{-1})**	**Nodule fresh weight (mg plant^{-1})**	**Shoot height (cm)**	**Root fresh weight (mg plant^{-1})**	**Shoot dry weight (mg plant^{-1})**	**Shoot nitrogen (mg plant^{-1})**
1	Control	Chillum jars	-	-	15.2	255.5	365.5	08.2
2	MR14		6	60.0	17.7	451.3	525.3	15.2
3	MR23		15	76.3	19.8	565.3	625.3	18.3
4	MS57		12	69.8	19.6	604.5	624.8	19.3
5	MB703		10	67.3	19.3	541.3	579.3	17.4
1	Control	Leonard jars	-	-	10.0	167.5	188.0	04.0
2	MR14		-	-	10.7	125.0	158.0	03.2
3	MR23		10	70.0	18.5	509.8	611.3	17.6
4	MS57		9	78.3	17.8	510.3	600.8	17.8
5	MB703		3	53.8	14.2	380.3	462.3	13.0
CD at 5%			3.0	11.5	2.1	24.5	29.1	3.2

Table.3 Efficacy of thermo tolerant rhizobial isolates in terms of nodulation and growth of mungbean under unsterilized conditions in pots

S. No.	Treatments	Nodule (number plant^{-1})	Nodule fresh weight (mg plant^{-1})	Shoot height (cm)	Root fresh weight (mg plant^{-1})	Shoot dry weight (mg plant^{-1})	Shoot nitrogen (mg plant^{-1})
1	Control	-	-	17.3	283.0	316.5	09.0
2	MR14	10	62.8	19.0	623.5	561.5	16.0
3	MR23	22	81.0	21.1	711.0	656.0	19.1
4	MS57	15	73.3	20.9	688.5	710.3	19.8
5	MB703	12	68.8	20.2	663.0	624.5	18.7
CD at 5%		5	12.5	1.5	28.3	33.5	2.6

Protein profile of thermo tolerant rhizobia

New proteins under high temperature conditions are known to be induced to make the cell thermotolerant. Protein profile of thermo tolerant rhizobia was assessed at higher and normal temperature of growth. To distinguish the differences in protein patterns one dimensional SDS PAGE was performed. Distinct differences in the whole cell protein patterns were observed between the rhizobial isolates under high temperature and normal temperature conditions. The SDS PAGE revealed that in mungbean rhizobial isolates, molecular weight of different polypeptides ranged from 18 to more than 116 kDa (Fig. 2). Comparative analysis showed that new polypeptide was present under high temperature stress (45ºC) conditions. Rhizobial isolates MR23 and MS57 showed the presence of new polypeptide bands compared to their respective controls grown under non stress conditions. New polypeptide bands of approximately 75 and 21 kDa were observed in the rhizobial isolate MR23 and of 20 kDa in the rhizobial isolate MS57 in response to high temperature stress (Fig.2).

Symbiotic effectivity of thermo tolerant rhizobia under different temperature regimes

Three temperature regimes were created by using chillum jars, Leonard jars and pots for studying the symbiotic efficacy of thermo tolerant mungbean rhizobia.. Temperature measurement at 10 cm depth showed that under sterilized conditions in chillum jars during the experimentation period temperature remained between 31 to 40ºC; while in Leonard jar 37 to 49ºC (Fig. 3). Under un sterilized conditions in pots temperature remained between 31.7 to 41ºC. Elevated temperature significantly affected the growth of mungbean as reflected in height of mungbean plants. Inoculation with all the four rhizobial isolates significantly increased the shoot height compared to the uninoculated control. Maximum soil temperature in chillum jars and pots reached to 40 and 41ºC respectively. Plants inoculated with thermo tolerant isolates MR23 and MS57 showed more height of 21.1 and 20.9 cm respectively in pots; 19.8 and 19.6 cm respectively in chillum jars than the plants inoculated with isolates MR14 and MB703 correspondingly 19.0 and 20.2

cm in pots and 17.7 and 19.3 cm in chillum jars (Table 2 and 3). Under the highest temperature regime of 49ºC in leonard jars, inoculation with thermo tolerant isolates MR23 and MS57 showed significantly higher shoot height of 18.5 and 17.8 cm respectively than the inoculation with reference strain MB703 with 14.2 cm. However, inoculation with MR14 did not show increase in shoot height under leonard jar conditions over uninoculated control (Table 2 and Fig 4).

A similar trend was reflected in the plant biomass as that of the shoot height (Table 2 and 3). Under the temperature regime of 41ºC in pots under un sterilized conditions, plants inoculated with thermo tolerant isolates MR23 and MS57 showed significantly more root biomass of 711.0 and 688.5 mg plant^{-1} and shoot biomass of 656.0 and 710.3 mg plant^{-1} respectively than the plants inoculated with thermo sensitive isolate MR14 (Fig 5 and Table 3). The lowest shoot height of 10.7 cm was observed when the plants were inoculated with isolate MR14 at 49ºC in leonard jars, similar trend in lowest shoot dry weight (158.0 mg plant^{-1}) and root fresh weight (125.0 mg plant^{-1}) was observed.

Under the temperature regime of 41ºC and 40ºC, thermo tolerant isolates MR23 and MS57 were more effective in forming nodules than isolates MR14 and MB703. In Leonard jars under the highest temperature regime of 49ºC, thermo tolerant isolates MR23 and MS57 showed better nodulation (10 and 9 nodules plant^{-1} respectively) whereas reference strain MB703 showed poor nodulation (3 nodules plant^{-1}) and MR14 did not form any nodule (Table 2, 3). On inoculation with rhizobial isolates MR23 and MS57, higher shoot nitrogen content of 19.1 and 19.8 mg plant^{-1} in pots and 18.3 and 19.3 mg plant^{-1} respectively in chillum jars was observed as compared to inoculation with MR14 and MB703 which showed 16.0 and 18.7 mg plant^{-1} in pots and 15.2 and 17.4 mg plant^{-1} respectively in chillum jars under the almost same temperature regime. Similarly, under the high temperature regime of 49ºC in Leonard jars, inoculation with rhizobial isolates MR23 and MS57 significantly improved the shoot nitrogen content to the extent of 17.6 and 17.8 mg plant^{-1} than the inoculation with isolates MR14 and MB703 which was 3.2 and 13.0 mg plant^{-1} respectively.

High temperature is detrimental to both rhizobial and host as symbiotic partners. Mungbean is less sensitive to temperature stress as compared to pigeonpea (Dudeja and Khurana, 1989b). In case of micro symbiont the rhizobia at higher temperature some modification of the symbiotic properties has been reported (Rennie and Kemp, 1986; Singh and Khurana, 1992). Further it has been reported that effect of root temperature on nodulation and dinitrogen fixation in legumes is modified by the strain of rhizobia (Arayangkoon et al., 1990). Different species and strains of rhizobia differ in their tolerance to high temperature (Karanja and Wood, 1988) and selection of strains for temperature tolerance has been suggested as a means of overcoming temperature stress. Therefore, tolerance to high temperature is a desirable property for rhizobial strains. Keeping this in view thermo tolerance of mungbean rhizobia in relation to their ability to fix dinitrogen in symbiotic association with mungbean under different regimes of temperature was envisaged in the present study.

Rhizobial isolates of mungbean which are usually more adapted due to natural environmental selection in the northern India where the temperature remains -2 to

47ºC and in 5cm soil depth it is even upto 50ºC (Dudeja and Khurana, 1989a). Mungbean nodules collected from farmer's fields of different districts of Haryana were used to isolate 91 rhizobial isolates. After authentication of the isolates by plant infection test, rhizobial growth at different temperatures showed that all rhizobia showed optimum growth between 30 to 40ºC and two isolates MR23 and MS57 showed good growth even at 45ºC. Predominately mesophilic rhizobia have optimum temperatures for growth in the range of 28-31°C. Maximum temperature for growth in free-living rhizobia ranged between 35-45°C (Zhang et al., 1991; Galvez, 2005). However, survival of rhizobial strains from *Sesbania aculaeta* ranged upto 50 and 65°C on YEMA plates for 2 to 4 h (Zhang et al., 1991). Further increase in temperature in mungbean rhizobia led to noticeable decline in growth. These findings agreed with the results of previous studies on *Rhizobium leguminosarum* strains isolated from Nile Valley of Egypt which showed tolerance to temperatures ranging between 35-40°C (Moawad and Beck, 1991) and *Cicer arietinum* rhizobial isolates, which grew at 45°C (Maatallah et al., 2002) similar to pigeon pea rhizobia (Zahran, 1999).

Further characterization of mungbean rhizobia showed decrease in EPS production observed with increase in temperature to 45ºC even in case of temperature tolerant rhizobial isolates MR23 and MS57. Similar results were observed with *Sinorhizobium* (Rasanen and Lindstrom, 1999). Colony morphology of *Sinorhizobium* changed from slimy to dry in some cases after heat stress treatment. Longer the rhizobial cells were affected by the heat stress, greater was the abundance of colonies with dry surface. However in case pigeonpea rhizobia at normal temperature of 30ºC, only half of the heat stress mutants of pigeon pea rhizobia produced higher amounts of EPSs than the parent strain, but at 43ºC, all the mutants produced higher quantities of EPS (Nandal et al., 2005). Change in the concentration of EPS may influence abiotic stress tolerance of the microorganisms.

Results of the present study showed that high temperature tolerant mungbean rhizobial isolates accumulated more trehalose when grown at higher temperature as compared to reference strains or at normal temperature. These results are consistent with trehalose accumulation kinetics observed for other bacteria such as pigeonpea (Nandal et al., 2005) and bradyrhizobia species (Elsheikh and Wood, 1990). It has also been suggested that trehalose could function as an osmo protectant in rhizobia and bradyrhizobia species under stress conditions. In addition to salinity stress and dessication, trehalose also protects bacterial cells from heat (McIntyre et al., 2007).

Abiotic stress provokes cessation of conventional protein synthesis accompanied by increased translation of stress related proteins (Michiels et al., 1994). These proteins have been described as highly conserved polypeptides which play an important role for survival both under normal and extreme conditions. Protein profile of thermo tolerant mungbean rhizobial isolates showed the expression of new proteins of different molecular weights at higher temperature as compared to protein profile at normal temperature. A protein band of approximately 21 and 20 kDa appeared in rhizobial isolates MR23 and MS57 respectively under high temperature (45ºC) stress conditions. Similarly, Michiels et al. (1994) compared the effects of heat on protein synthesis in bean-nodulating *Rhizobium* strain and found a

heat shock protein of approximately 21 kDa whose synthesis was strongest in both the *Rhizobium* strains upon a temperature shift up. Similarly in pigeonpea rhizobia the protein electrophoretic pattern showed that the parent strain PP201 formed very few proteins at high temperature, whereas the mutants formed additional new proteins (Rasanen and Lindstrom, 1999). A heat shock protein of 63–74 kDa was overproduced in all mutant strains. Laranjo and Oliveira (2011) observed a high diversity in tolerance to temperature stress among *Mesorhizobium* species. *Mesorhizobium plurifarium* showed highest growth at 37°C. SDS PAGE analysis revealed changes in their protein profiles. Several proteins overproduced in different strains may be involved in stress tolerance.

Soil temperature affected both the symbionts of pigeonpea under these conditions (Dudeja and Khurana, 1988c, 1989a, b) may increase at seeding time and during the period of plant growth. In each instance, high temperature may be deleterious. In the present study, temperature significantly influenced the growth and nodulation of mungbean as there was increase in temperature from 40 to 49ºC. Aranjuelo et al. (2007) evaluated the effect of elevated temperature on the plant growth of alfalfa and observed reduction in plant weight by the high temperature. Similarly, Dahiya et al. (1981) reported reduction in the shoot weight and nitrogen in pigeonpea at elevated temperatures in leonard jars as compared to chillum jars. High temperature above 35ºC decreased nodule weight and number, nitrogenase activity and shoot dry matter production in pigeonpea and soybean (Lindermann et al., 1974; Dart et al., 1976).

In the present study, two rhizobial isolates namely MR23 and MS57 were capable of forming nodules even at 49ºC under sterilized leonard jars conditions while MR14 did not form any nodule. Rhizobia growing in soils in India during the summer season are subjected to high temperature stress. Furthermore, surface temperature in soils in this region often rises to 50°C. The ability of a population to survive during these periods would ensure their presence in high numbers in the following season. Rhizobia investigated were quite resistant to such high temperature. Differences in temperature response among the isolates point to the potential failure of rhizobial isolate MR14 to be used as inoculant in regions with high temperatures to which the bacteria are not adapted. Rhizobia are more resistant to high temperatures in soil than in laboratory medium. In the light of present results, it may be concluded that high temperature stress in present investigation negatively affected all parameters of growth such as root and shoot growth, nodule number, nodule weight and shoot nitrogen content in mungbean plants. However, ability of mungbean to grow and survive under temperature stress conditions was improved when it was inoculated with thermo tolerant isolates of mungbean rhizobia. Further work is required to observe the survival, competitiveness, nodule formation and crop productivity under field conditions so that these inoculants could be recommended for biofertilizers production.

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