Characterization of heat resistant mutant strains of *Rhizobium* sp. [*Cajanus*] for growth, survival and symbiotic properties

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Abstract Fourteen heat resistant mutant strains were isolated from a wild-type strain (PP201, Nod⁺ Fix⁺) of *Rhizobium* sp. (*Cajanus*) by giving it a heat shock of 43°C. These mutant strains showed a greater increase in optical density (O.D.) and a higher viable cell count in both rhizospheric and non-rhizospheric soil at high temperature. Symbiotic studies showed that pigeon pea plants inoculated with a few mutant strains had ineffective nodules (Nod⁺ Fix⁻) under controlled temperature (43°C) conditions, but under natural high temperature (40–45°C) conditions, the host plants infected with all the mutant strains showed higher total shoot nitrogen than the plants inoculated with the parent strain. Four mutant strains (HR-3, HR-6, HR-10 and HR-12) were found to be highly efficient for all the symbiotic parameters, and thus have the potential to be used as bioinoculants in the North-Western regions of India during the summer season.

Keywords Heat resistant mutants · Growth · Survival · *Rhizobium* sp. (*Cajanus*) · Symbiotic properties

Introduction

High soil temperature in North-Western parts of India is a major constraint for biological nitrogen fixation in legume crops. In these areas and other tropical regions, the temperature in the upper 5–6 cm layer of the soil reaches up to 40–50°C during the summer season and the inoculants are exposed to these elevated temperatures for varying lengths of time^{1,2}. Such high temperatures in the surface soils have adverse effect on rhizobial growth, survival, establishment in the soil and on the process of symbiosis itself^{3,4}. If the exposure period to high temperature is prolonged, it results in partial or sometimes even complete elimination of rhizobia. Therefore, the complete benefit of the legume-*Rhizobium* association can not be fully realized when soil temperatures become limiting. Hence, rhizobial strains to be used as bioinoculants in the areas witnessing increased temperatures during the summers must have some degree of resistance/tolerance to high temperature.

Pigeon pea [*Cajanus cajan* (L.) Millsp.] is one of the major kharif legumes of the tropics ranking sixth among different pulses in respect of area and production⁵. Of the various factors affecting pigeon pea productivity, poor nodulation capacity of the legume under high temperature stress is a major bottleneck in harnessing the beneficial effects of biological nitrogen fixation. High soil temperature not only affects the survival and persistence of *Rhizobium* inoculants in pigeon pea rhizosphere⁶ but also has an adverse effect on several aspects of *Rhizobium*-legume symbiosis, including the root hair formation, adsorption of rhizobia, nodulation, nitrogenase enzyme activity and consequently the nitrogen content and dry matter production of the nodulated host plant⁷. Therefore, for full exploitation of the pigeonpea-*Rhizobium* symbiotic system, development of temperature

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resistant/ tolerant strains for use as biofertilizers has been suggested as a means of overcoming temperature stress. The present investigation was therefore, aimed at isolating heat resistant mutant strains of *Rhizobium* sp. (*Cajanus*) and characterizing these mutants for their growth, survival and symbiotic properties at high temperature under both controlled and natural conditions.

Materials and Methods

Bacterial strains and culture medium: The wild-type strain PP201 of *Rhizobium* sp. (*Cajanus*) used in the present study was obtained from the Department of Microbiology, CCS HAU, Hisar. The medium used for general culturing and for isolation of mutant strains was yeast extract mannitol agar (YEMA)⁸.

Isolation of heat resistant mutants and their growth characteristics at elevated temperature: For isolation of heat resistant mutants, log phase culture of the wild-type strain (PP201) was subjected to a heat-shock for varying time intervals (1, 3, 5, 6, 8, 10 and 12 h) at 43, 44 and 45°C. The culture was then taken out after respective time intervals, dilution plated on YEMA medium and incubated at both normal (30°C) and high temperature (43–45°C). The colonies showing stable growth at high temperature on YEMA medium after repeated rounds of streaking and restreaking were adjudged as heat resistant/tolerant mutants.

Growth characteristics of the isolated mutants were studied in both solid and liquid medium. The wild-type strain and heat resistant/tolerant mutants were spotted onto YEMA plates (in triplicate) and then incubated at different temperatures (30, 35, 40, 41, 42, 43, 44 and 45°C). The growth of the mutants was compared with that of the parent strain after 3–4 days of incubation. Similarly, the growth of mutant strains was studied in YEM broth. A loopful of cell suspension of the wild-type and mutant strains was inoculated into YEM broth and incubated at 30°C till log phase was reached. One ml of this starter culture was then inoculated into 100 ml of fresh sterile YEM broth. Three flasks of each strain were incubated at 30°C and 43°C and samples were withdrawn from the cultures at regular intervals of time viz. 0, 6, 12, 24, 48, 72, 96 and 120 h. Growth was measured as increase in optical density (O.D.) at 600 nm.

Survival of wild-type strain and its mutants in sterilized rhizospheric and non-rhizospheric soil at high temperature: For determination of viable cell count in the rhizosphere, samples were taken from previously sterilized rhizospheric soil closely adhering to the roots of the plants which were inoculated with the parent strain and its mutants and raised

under controlled conditions comprising a daily heat-shock of 6 h at 43°C in a plant growth chamber. Non-rhizospheric count was determined by inoculating 10 ml log phase culture of each strain into chillum jars⁹ containing 500 g of sterilized sand. These chillum jar assemblies (in triplicate) were then kept under controlled conditions (daily heat shock for 6 h at 43°C and 30°C for rest of the day). One-gram soil samples were taken from both rhizospheric and non-rhizospheric soil after different time intervals i.e. 0, 15, 30 and 45 days. Appropriate dilutions of these samples were plated on YEMA (Congo red) medium and the plates incubated at 30°C. Viable cell count was calculated by counting the number of colonies that appeared on different dilution plates after 4–5 days of incubation.

Symbiotic characterization of wild-type strain and its mutants: Symbiotic performance of the wild-type strain and its mutants was tested on pigeon pea plants grown in chillum jars containing sterilized sand under controlled conditions in a plant growth chamber and also in pots in the net house.

a. Under controlled conditions: Surface sterilized seeds were inoculated with cultures of wild-type/mutant strains and five inoculated seeds were sown in each chillum jar. These chillum jars were then kept under controlled conditions in the plant growth chamber. After germination of seeds, thinning was done to retain three seedlings per chillum jar. The plants were subjected to a daily heat-shock of 6 h at 43°C and thereafter, the air temperature was maintained at 30°C with a photoperiod of 16 h/day. These temperature conditions were standardized after conducting a series of experiments in which the host plant was subjected to a daily heat shock of 43°C for variable time periods. The plants were uprooted 50 days after sowing (DAS) and the following symbiotic parameters were recorded: nodule number, nodule fresh and dry weight, and root and shoot dry weight. The total shoot nitrogen was determined by micro-Kjeldahl's method¹⁰.

b. Under natural conditions: Symbiotic efficiency of the parent strain and the mutants was also tested on host plants grown in earthen pots containing sterilized sand and kept in the net house. There were three replications for each strain and three plants per pot. The plants were alternately supplied with sterilized N₂-free Sloger's solution¹¹ and sterile distilled water. The experiment was conducted during the months of June–July, with day-time temperature varying between 40–45°C. Fifty days after sowing, the plants were uprooted from the sand and the data on nodule fresh and dry weight, and root and shoot dry weight was recorded. The total shoot nitrogen was determined by micro-Kjeldahl's method¹⁰.

Results and Discussion

Isolation and growth pattern of heat resistant/tolerant mutants: Fourteen heat resistant/tolerant mutant strains were isolated from the wild-type strain PP201 after giving it a heat shock treatment of 43°C for 6 h. These isolates growing well at 43°C were designated as HR-1 to HR-14. Heat shock treatment at 44 and 45°C failed to yield any stable mutant. Use of heat treatment for the isolation of mutants has been reported in a few earlier studies too. Different species and strains of rhizobia differ in their tolerance to high temperature¹² and studies have been conducted to examine the growth response of different rhizobium strains to increasing temperatures in order to identify temperature-related growth characteristics that can be used to select temperature-tolerant strains¹³. In temperature tolerant studies conducted by Purushothaman and Vijila (1988)¹⁴, 15 high temperature resistant strains were isolated by growing wild-type *Azospirillum* strain for two weeks at 50°C. More recently, Gopalakrishnan and Dudeja (1999)¹⁵ have reported the selection of a thermo-tolerant variant of a rhizobial strain capable of growing at 43°C by progressively increasing the inoculum's temperature. In contrast to these studies, mutants in the present investigation were isolated by subjecting the wild-type parent strain to an initial brief heat shock of 6 h at 43°C (instead of a continuous heat treatment for longer duration) and then purifying the isolates by repeated rounds of incubation at 43°C.

When all the mutant strains were tested for their ability to survive and grow at various high temperatures on YEMA medium, it was found that at 30 and 35°C, the parent strain as well as the mutant strains showed good growth. However, upon increasing the temperature to 43°C, only the mutant strains showed growth, while the parent strain could not tolerate this temperature. Based on the intensity of the growth of the mutants at 43°C, these were categorized into two groups viz. heat resistant showing good growth at 43°C (HR-1 to HR-12) and heat tolerant showing reduced growth at 43°C (HR-13 and HR-14).

In YEM broth, the parent strain and the mutants showed comparable growth at normal temperature (30°C) but the growth was highly variable at 43°C. The parent strain showed an increase in O.D. only upto 6 h of incubation at elevated temperature (43°C), but the mutant strains at 43°C showed an increase in O.D. upto 96 h of incubation, after which the O.D. became almost constant. The fold-increase in O.D. after 120 h of incubation at 30°C was almost similar in the parent strain and the mutants (except HR-6 which showed an exceptionally high increase). However, at 43°C, the parent strain showed only a 3-fold increase in O.D, whereas the mutants showed an increase in the range of 8.0 to 20.0-folds, the fold-increase being lower for the heat tolerant than the heat resistant mutants (Fig. 1). The maximum fold-increase in O.D. at 43°C was shown by HR-6 (20.00-fold increase). Although the mutant strains showed good growth at high temperature, but their mean generation time was still found to be high at 43°C. This observation is

Fig. 1 Fold increase in O.D. of the parent strain (PP201) and heat resistant/tolerant mutants in YEM broth after 120 h of incubation at 30°C and 43°C.

in conformity with the results obtained by Hashem *et al.* (1998)¹⁶ in thermotolerant *Lucaena*-nodulating *Rhizobium* strains. Differences in the growth pattern and survival rate of different species of *Rhizobium* in liquid medium at elevated temperatures have been used to categorize the strains into temperature-tolerant or temperature-sensitive strains^{17, 18}.

Viable cell count of the parent strain and its mutants in sterilized rhizospheric and non-rhizospheric soil under controlled conditions: A marked difference was observed between the survival of the parent strain and the mutants in the soil under heat stressed conditions (43°C for 6 h day⁻¹), both in the rhizospheric and non-rhizospheric soil (Table 1). The decrease in number of cells g⁻¹ soil after 45 days of inoculation was much less in case of mutants than the parent strain in both rhizospheric and non-rhizospheric soils. The parent strain showed a decrease from 10⁸ to 10² cells g⁻¹ soil after 45 days, while the mutants showed a decrease from 10⁸ cells to 10⁵ cells g⁻¹ soil. The per cent decrease after 45 days of inoculation in parent strain was 65.09, whereas for mutants it was in the range of 27–32 % in the rhizospheric

soil and 33–42% in the non-rhizospheric soil, indicating the higher multiplication rate of the mutant strains at high temperature conditions. Heat tolerant mutants (HR-13 and HR-14) showed a slightly higher per cent decrease (40–48%). From these observations, it is clear that the mutants could tolerate high temperature and consequently showed better survival in the soil (both rhizospheric and non- rhizospheric) as compared to the parent strain under high temperatures.

Symbiotic properties of parent strain and its mutants in controlled and natural high temperature conditions:

a. Under controlled conditions: The data on symbiotic properties with cyclic temperature regime of 43°C for 6 h (Table 2) indicate that out of 14 mutant strains, 8 mutants (HR-1 to HR-4, HR-6, HR-8, HR-10 and HR-12) induced nodules on pigeon pea plants (Nod⁺), but the parent strain did not cause nodulation (Nod⁻) on the host plants at this temperature. However, the number of nodules formed by the pigeon pea plants inoculated with the mutants was very low. The host plants infected with only two mutant strains (HR-1 and HR-6) formed more than five nodules per plant.

Table 1 Viable cell count of the parent strain (PP201) and heat resistant/tolerant mutants in the rhizospheric and non-rhizospheric soil of pigeon pea plants subjected to a daily heat shock of 6 h at 43°C.

Strain/Mutant	Viable cell count (No. of cells g soil ⁻¹)							
	0 Day		15th Day		30th Day		45th Day	
	Rhizo-spheric	Non-rhizospheric	Rhizo-spheric	Non-rhizospheric	Rhizo-spheric	Non-rhizospheric	Rhizo-spheric	Non-rhizospheric
Uninoculated Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PP201 (Parent)	3.32 × 10 ⁸	14.40 × 10 ⁸	3.49 × 10 ⁶ (23.21)	2.73 × 10 ⁶ (29.72)	8.45 × 10 ⁴ (42.18)	29.8 × 10 ⁴ (40.23)	9.45 × 10 ² (65.09)	19.00 × 10 ² (64.20)
Mutants Heat resistant	(× 10 ⁸ cells g soil ⁻¹)		(× 10 ⁷ cells g soil ⁻¹)		(× 10 ⁶ cells g soil)		(× 10 ⁵ cells g soil ⁻¹)	
HR-1	3.89	5.43	2.42 (14.04)	1.53 (17.74)	3.70(23.54)	6.81(21.77)	5.81(32.90)	4.44(35.35)
HR-2	1.00	4.04	1.64 (09.83)	3.56 (12.26)	6.02(15.25)	7.90(19.85)	4.66(29.15)	5.60(33.21)
HR-3	1.20	5.93	4.09 (05.78)	2.87 (15.00)	7.64(14.80)	8.15(21.22)	7.56(27.23)	4.80(35.24)
HR-4	1.55	3.03	1.41 (12.71)	3.15 (11.59)	4.75(18.47)	5.22(20.79)	4.58(30.88)	2.13(37.18)
HR-5	1.20	3.33	4.25 (05.58)	2.33 (13.55)	5.30(16.77)	3.67(22.96)	5.43(29.01)	2.67(36.32)
HR-6	3.90	4.90	3.38 (12.36)	1.62 (17.03)	6.69(20.56)	2.69(26.01)	7.16(31.85)	3.48(36.23)
HR-7	2.21	4.61	2.17 (12.08)	4.03 (12.22)	4.04(20.83)	1.54(28.58)	5.67(31.04)	3.41(36.14)
HR-8	3.22	2.87	1.18(16.88)	3.77 (10.43)	5.21(21.03)	2.82(23.74)	6.90(31.37)	2.80(35.60)
HR-9	2.10	5.55	1.22 (14.85)	2.48 (15.44)	4.19(20.43)	1.30(30.08)	4.95(31.57)	3.09(37.21)
HR-10	2.11	7.45	1.44 (14.08)	1.01 (21.06)	3.72(21.06)	1.85(29.36)	6.80(29.93)	1.22(42.67)
HR-11	1.13	5.58	1.22 (12.01)	4.27 (12.77)	2.79(19.96)	1.85(28.35)	2.99(32.00)	1.77(40.00)
HR-12	3.75	7.30	4.10 (11.21)	4.63 (13.51)	7.10(20.10)	2.52(27.78)	8.67(30.74)	3.02(38.17)
Heat tolerant								
HR-13	2.06	4.60	1.36 (14.19)	1.21 (18.24)	0.90(28.40)	2.30(26.57)	0.67(42.00)	1.83(39.26)
HR-14	1.79	6.02	1.12 (14.59)	0.32 (25.91)	0.74(28.91)	1.04(31.49)	0.56(42.47)	0.30(48.99)

Values in parentheses indicate % reduction in rhizobial cells

Table 2 Symbiotic properties of the parent strain (PP201) and its mutants under controlled conditions (daily heat shock of 6 h at 43°C).

Strain/Mutant	Nodule no. plant ⁻¹	Nodule fresh weight (mg plant ⁻¹)	Nodule dry weight (mg plant ⁻¹)	Root dry weight (mg plant ⁻¹)	Shoot dry weight (mg plant ⁻¹)	Per cent shoot nitrogen	Total shoot nitrogen (mg N ₂ plant ⁻¹)
Uninoculated (control)	0.00	0.00	0.00	38.33	99.67	1.27	1.27
PP201 (Parent)	0.00	0.00	0.00	48.67	130.0	1.93	2.50
Mutants Heat resistant							
HR-1	5.33	71.67	8.33	81.33	148.33	2.02	2.99
HR-2	3.00	26.00	4.00	39.33	121.33	2.11	2.56
HR-3	4.00	29.00	5.00	75.00	136.00	2.02	2.75
HR-4	3.00	22.00	3.00	43.00	125.67	1.98	2.49
HR-5	0.00	0.00	0.00	50.67	136.67	2.14	2.92
HR-6	6.00	73.00	9.00	58.00	132.00	2.01	2.65
HR-7	0.00	0.00	0.00	50.67	115.33	1.96	2.26
HR-8	4.00	56.00	6.00	51.67	136.67	2.03	2.77
HR-9	0.00	0.00	0.00	51.67	134.00	2.07	2.77
HR-10	4.00	41.00	5.00	65.00	132.00	2.06	2.83
HR-11	0.00	0.00	0.00	52.00	116.33	2.08	2.42
HR-12	4.00	33.00	4.00	59.67	127.67	2.01	2.57
Heat tolerant							
HR-13	0.00	0.00	0.00	52.67	110.00	1.96	2.15
HR-14	0.00	0.00	0.00	53.00	107.33	1.94	2.08
C.D. at 5% level	1.13	7.87	1.94	14.83	18.50	0.11	0.40

The plants inoculated with HR-1, HR-6 and HR-8 had maximum nodule fresh and dry weights. In spite of poor nodulation, a positive effect of inoculation was observed on root dry weight. The pigeon pea plants inoculated with the mutant strains (except HR-2 and HR-4) showed higher root dry weight than the plants infected with parent strain, the increase being significant for HR-1 and HR-3. The per cent shoot nitrogen of host plants infected with five mutant strains HR-2, HR-5 and HR-9 to HR-11 was slightly higher than the parent strain, but the total shoot nitrogen of the host plants inoculated with HR-1 and HR-5 was significantly higher.

Under controlled conditions, only a few mutant strains induced nodules on the host plants and these were mostly ineffective (Nod⁺Fix⁻). The reason for this poor nodulation can be ascribed to the fact that high temperature has an adverse effect not only on the bacterial strain, but also on the plant metabolism. These results are in conformity with those obtained by many other workers. It has been reported that with constant high temperature (35°C or more) treatment, either there is no nodulation¹⁹ or the nodules are ineffective²⁰. But,

similar to our results, the diurnal cyclic temperature regimes like 41°C for 6 or 9 h day⁻¹ have shown to cause nodulation on the host plants by all the strains of bradyrhizobia²¹. Moreover, Hungria *et al.* (1993)²² have observed that when common beans inoculated with certain rhizobial strains (which were capable of fixing nitrogen at high temperatures) were exposed to high diurnal temperature conditions of 40°C for 8 h day⁻¹; some of these strains, in addition to causing nodulation, also induced accumulation at flowering time as much or more nitrogen as bean plants receiving mineral nitrogen. Similarly, Hashem *et al.* (1998)¹⁶ have reported that when symbiotic studies were carried out in *Lucaena*-nodulating rhizobial strains capable of growing at 42°C, 50 per cent of these high temperature tolerant strains were found to be efficient in fixing nitrogen at diurnal temperature conditions consisting of 42°C for 14 h day⁻¹ and 25°C for the rest of the day. The remaining 50 per-cent tolerant strains were relatively ineffective (Fix⁻) although these formed several nodules at high temperatures (Nod⁺).

b. Under natural high temperature conditions: The data on symbiotic characteristics under natural high temperature

(40–45°C) conditions (Table 3) in the net house show that all the mutant strains nodulated (Nod⁺) the host plants. The plants inoculated with the mutant strains HR-3, HR-6, HR-10 and HR-12 showed significantly higher nodule fresh and dry weight than the plants inoculated with the parent strain. The maximum root and shoot dry weights were observed in pigeon pea plants inoculated with the mutant strain, HR-6. Significantly higher values of above parameters were also observed in plants infected with the mutants HR-3, HR-10 and HR-12 than the parent strain. The per cent and total shoot nitrogen were also significantly higher in the plants inoculated with the mutants HR-3, HR-6, HR-10 and HR-12, the maximum being observed in mutant strain HR-12. The two heat tolerant mutant strains HR-13 and HR-14 were less effective on the host plants than the heat resistant mutants. Thus, the observations on symbiotic properties indicate that the four mutant strains (HR-3, HR-6, HR-10 and HR-12) had high symbiotic effectivity under net house conditions. The HR-12 mutant strain has been shown to possess significantly higher symbiotic performance than the pigeon pea plants inoculated with the parent strain in field conditions²³. These results suggest that the adverse effect of the root temperature on plant development can be moderated

by specific temperature-tolerant strains of *Rhizobium*. A few earlier studies have also reported that a correlation exists between the ability of *Rhizobium* strains to grow at high temperatures and their ability to nodulate the host plants^{24,25}. Karanja and Wood (1998)²⁴, while screening several strains of *Rhizobium japonicum* for their ability to multiply at high temperatures, obtained a few strains tolerant to 40°C. Four such strains were found to remain infective even after incubation at 40°C, suggesting that these high temperature-tolerant strains were useful for enhancing nodulation and nitrogen fixation. In another study conducted by Munevar and Wollum²⁵ on soybean plants inoculated with thirteen different strains of *Rhizobium japonicum*, it was found that the magnitude of the effect of increasing root temperatures from 28 to 40°C on nodulation, nitrogen fixation and dry matter production of soybean plants was highly dependent on the response of rhizobial strains to temperature in pure culture.

The reason for better symbiotic performance of heat resistant/tolerant mutants obtained in this study over the parent strain under natural high temperature conditions and also the nodulation ability of some of the mutants under the diurnal temperature regime of 43°C for 6 h is not due

Table 3 Symbiotic properties of the parent strain (PP201) and heat resistant/tolerant mutants under natural high temperature (40–45°C) conditions.

Strain/mutant	Nodule fresh weight (mg plant ⁻¹)	Nodule dry weight (mg plant ⁻¹)	Root dry weight (mg plant ⁻¹)	Shoot dry weight (mg plant ⁻¹)	Per cent shoot nitrogen	Total shoot nitrogen (mg N ₂ plant ⁻¹)
Uninoculated (control)	0.00	0.00	91.70	323.00	1.13	3.64
PP201 (Parent)	138.10	33.33	109.57	411.33	2.05	8.45
Mutants Heat resistant						
HR-1	168.63	50.97	139.77	495.33	2.43	12.05
HR-2	164.10	51.43	183.10	477.00	2.38	11.35
HR-3	286.80	92.23	231.90	572.67	2.91	16.78
HR-4	136.57	42.23	155.67	368.00	2.45	9.01
HR-5	140.43	54.00	164.90	438.33	2.52	11.03
HR-6	222.10	68.00	279.77	698.00	2.60	18.22
HR-7	126.33	39.33	152.43	470.67	2.49	11.69
HR-8	159.23	57.67	187.23	421.00	2.73	11.43
HR-9	127.43	42.63	133.77	451.33	2.45	11.05
HR-10	255.87	76.80	188.10	578.00	2.63	15.17
HR-11	146.30	34.67	170.77	446.67	2.32	10.31
HR-12	218.67	78.10	219.23	679.33	3.07	20.78
Heat tolerant						
HR-13	151.43	50.77	169.13	505.67	2.15	10.81
HR-14	121.47	29.33	128.87	312.67	1.96	6.13
C.D. at 5% level	45.79	15.31	49.61	102.48	0.31	3.21

to the ability of the mutants to fix atmospheric nitrogen at high temperature, but it is the high temperature tolerating capacity of the mutant strains which allows them to survive the periods of thermal stress and then to recover afterwards. This can be corroborated by the fact that all these mutant strains strongly overproduced three high molecular weight proteins of 100, 89 and 64–72 kDa and several other small molecular weight proteins (14–29 kDa) at high (43°C) temperatures²⁶. These proteins might be responsible for imparting heat resistance or tolerance to the mutants.

In conclusion, this study shows that heat shock treatment can lead to the isolation of heat resistant/tolerant mutants of *Rhizobium* sp. (*Cajanus*) which can tolerate thermal stress and can fix atmospheric N₂ more efficiently than the parent strain under natural high temperature conditions. Since resistance to temperature stress constitutes a prerequisite for symbiotic nitrogen fixation in areas witnessing elevated temperatures, the heat resistant/tolerant mutant strains isolated in the present study have the potential to be used as biofertilizers in the high temperature conditions prevailing during summers in the North-Western parts of India.

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