

Evaluation of Thermotolerant Mungbean Rhizobial Isolates for Plant Growth Promoting Traits

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ABSTRACT

Thermotolerant rhizobial isolates retrieved from root nodules of *Vigna radiata* grown at CCSHAU Hisar farm were evaluated for various plant growth promoting traits. Among four thermotolerant rhizobial isolates screened for PGP traits, the isolate MRH 59 showed high IAA production, ammonia excretion, HCN production, Siderophore production and phosphate solubilisation. The promising rhizobial isolate exhibiting multiple plant growth promoting traits and showing nodulation of summer mungbean under sterilised conditions can be exploited for growth promotion of summer mungbean.

Keywords: HCN production, IAA production, mungbean, P-solubilization, *Rhizobium*

INTRODUCTION

Mungbean (*Vigna radiata*) is one of the important legumes and a well-known monetary crop in tropical and sub-tropical countries. It is highly nutritious crop and is regarded as a quality pulse due to its rich protein content (24%) and excellent digestibility. It is the third most important pulse crop in India, occupying nearly 3.72 million ha area with 1.56 million tonnes production. India is the largest producer and consumer of mungbean and accounts for about 65% of the world acreage and 54% of the world production. Total production of mungbean during the year 2016-2017 was 20.70 lakh tones, contributing 9.2% in total pulse production (Annual report 2016-17).

Mungbean is short duration crop of 60-70 days. It is usually included in rice or corn based crop rotation to replenish nitrogen

and improve soil fertility (<http://agricoop.nic.in>). There are lot of problems encountered in mungbean cultivation. Climatic conditions like high temperature during summer greatly affect the crop. Disease is another biological factor which constrains the productivity of mungbean crop as this crop is vulnerable to bacterial, viral and fungal diseases. Biofertilizers are applied for various plant growth promoting traits to solve some of these problems. Biofertilizers are known to increase the crop productivity by increasing the supply or availability of primary nutrients to the host plant. The use of biological nitrogen fixation (BNF) technology in the form of *Rhizobium* inoculants in grain legumes can be an alternative to expensive fertilizer, particularly for improving the production of food legumes in the country.

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Yield increases in mungbean by 10 to 37% following *Rhizobium* inoculation have been reported by many researchers (Kothari & Saraf, 1987). In addition to symbiotic nitrogen fixation, *Rhizobium* can also produce phytohormones, siderophores, HCN; thereby reducing the threat of pests and diseases and ultimately improve plant growth and yield of legumes (Manasa et al., 2017). They can solubilize sparingly soluble organic and inorganic phosphates, and they can colonize the roots of many non-legume plants. Rhizobia have a good potential to be used as biological control agents against some plant pathogens (Datta et al., 2015). The present investigation was carried out to select thermotolerant rhizobia exhibiting plant growth promoting traits to be used as biofertilizer for summer mungbean crop.

MATERIALS AND METHODS:

Thermotolerant rhizobial isolates retrieved from mungbean nodules and growing upto 45°C (Monika and Wati, 2017) were evaluated for various plant growth promoting traits under *in vitro* conditions.

Indole acetic acid (IAA) production

Thermotolerant rhizobial isolates were tested for their ability to produce indole acetic acid (IAA) using standard method (Gordon & Weber, 1951). Cultures were inoculated in 30 ml Yeast Extract Mannitol broth supplemented with DL-tryptophan at the rate of 100 µg/ml and were incubated at different temperatures (30, 35, 40 & 45°C) for eight days under stationary conditions of growth. Culture samples were withdrawn after regular interval of two days till 8th day and centrifuged at 10,000 rpm for 15 min. Two ml of Salkowski reagent was added to 2 ml of culture supernatant, mixed and allowed to stand for 30 min for the development of pink colour and colour intensity was estimated at 500 nm using spectrophotometer against a reagent blank.

Ammonia excretion

To screen the rhizobial isolates for their ability to excrete ammonia, all thermotolerant rhizobial isolates were grown in duplicate in 5ml peptone broth in 50ml flasks. The flasks were incubated at

30°C for 4 days under stationary conditions. Two ml culture broth was withdrawn at a regular interval of 2 days till 4 days and centrifuged at 10,000rpm for 15min. Ammonia released in the supernatant was determined by using the method of Chaney and Marbach (1962).

Siderophore production

Siderophore production by rhizobial isolates was detected by CAS (Chrome azurol S) assay (modified method of Schwyn and Neilands, 1987). Five µl inoculant of each log phase grown culture was spotted on Chrome azurol S (CAS) agar medium plates and incubated at different temperatures (30, 35, 40 and 45°C) for 3-4 days. The production of siderophore was indicated by the decolourization of blue-coloured ferric dye complex, resulting in yellow halo zones around the colonies.

Hydrogen cyanide production

Thermotolerant rhizobial isolates were screened for hydrogen cyanide production in King's B broth amended with glycine (4.4 g/l). Inoculum was prepared in YEM broth and 48 h culture from YEM broth was transferred to freshly prepared King's B broth. The production of cyanide was detected after 72 h inoculation at different temperatures (30, 35, 40 & 45°C), using picrate/ Na₂CO₃ paper fixed underside the test tube (Alstrom & Burns, 1989). A change from yellow to brown, brown and reddish brown colour was recorded as indication of weak, moderate or strong cyanogenic potential.

Phosphate solubilization

For measurement of phosphate solubilization activity, 10µl of active culture of each thermotolerant rhizobial culture grown in YEM broth was spotted on Pikovskaya's plate containing tricalcium phosphate (Pikovskaya, 1948) and incubated at different temperatures (30, 35, 40 & 45°C) for 7 days. The plates were observed for clear phosphate solubilization zone around colonies. The halo size produced by respective rhizobia was calculated according to the formula:

Solubilization Index = $\frac{\text{Zone diameter (mm)} + \text{colony diameter (mm)}}{\text{colony diameter (mm)}}$

RESULTS AND DISCUSSION

Abiotic stress in the form of high temperature severely affects the growth, nodulation and yield of mungbean. The use of thermotolerant rhizobia exhibiting plant growth promoting traits may prove useful in developing strategies to facilitate plant growth under normal as well as diverse abiotic stress conditions.

Indole-acetic-acid (IAA) being the major and most abundant auxin in plants, plays a key role in plant growth regulation and development. Many PGPRs have the ability to produce IAA equivalents and remarkably affect the plant growth by altering the endogenous level of auxin synthesized in plants. In present study, indole acetic acid production (IAA) by different rhizobial isolates ranged between 23.15 to 58.83 µg/ml in the presence of 0.01% tryptophan at 30°C on 5th day (Table 1). Production of IAA by both rhizobial and non rhizobial bacterial isolates decreased with increase in temperature (Table 2-4). Maximum IAA production was recorded in MRH59 *i.e.* 58.83, 56.58, 54.77 and 39.86 µg/ml at 30, 35, 40 and 45°C respectively. Different researchers have reported different temperature for IAA production by different rhizobia. According to Sudha *et al.*, (2012) optimum temperature for IAA production by *Rhizobium* sp. was 37°C. Modi and Khanna (2018), reported that pigeonpea rhizobial isolates showed increased IAA production at 40°C, whereas, all the isolates showed a sharp drop in IAA production at 50°C.

The ability of thermotolerant rhizobial isolates to excrete ammonia, varied from 3.65 to 4.78 µg/ml at 30°C and the efficiency of all isolates to excrete ammonia decreased with increasing temperature and the value ranged between 3.42 to 4.50 µg/ml at 35°C, 3.20 to 4.39 µg/ml at 40°C and 1.89 to 3.32 µg/ml at 45°C (Table 1-4). Maximum ammonia excretion was observed in isolate MRH59 *i.e.* 4.78, 4.50, 4.39 and 3.32 µg/ml at 30, 35, 40 and 45°C respectively, followed by MRH 46 *i.e.* 4.34, 4.14, 4.11 and 2.91 µg/ml at 30, 35, 40 and 45°C respectively. Different workers have reported different level of ammonia

excreted by different rhizobial species. Monika and Wati (2017) tested eleven rhizobial isolates for ammonia excretion and found that most of the isolates were able to excrete ammonia, which varied from 0.92 to 3.70 µg in liquid medium after 4 days of incubation. Siderophores also play significant role in microbial infection and the antagonism against plant pathogens. The production of siderophores by microorganisms is valuable to plants, because it can inhibit the growth of plant pathogens. Therefore, siderophore production by different rhizobial isolates was assessed in solid medium under *in vitro* conditions. Different rhizobial isolates were showing good or moderate growth in CAS agar plates incubated at 30 and 35°C (Table 1, 2). With increase in temperature to 40 to 45°C the efficiency of siderophore production decreased as indicated by retarded growth (Table 3, 4). MRH59 was showing good growth at 30, 35 and 40 °C but moderate growth at 45°C. Ahemad and Khan (2012) observed that *Rhizobium* sp. strain MRP1 displayed siderophores-producing potential by forming an orange zone of 11 mm size on pesticide free CAS agar medium.

HCN production is an important plant growth promoting trait which indirectly influences plant growth. By synthesizing HCN some rhizobia prevent the occurrence of plant diseases by providing strength to the host's disease resistance mechanism. Among selected thermotolerant rhizobial isolates, MRH 59, MRH 46 were high producers of HCN at 30 and 35°C (Table 1&2). Efficiency to produce HCN by rhizobial isolates decreased with increase in temperature but it was still high in MRH59 and MRH46 at 40°C and moderate at 45°C. Manasa *et al.* (2017) reported that out of 15 *Rhizobium* isolates, 8 produced HCN and out of 8 isolates RR-1 exhibited strong HCN production and GNR-1 scored as moderate for HCN production. Adnan *et al.* (2016) isolated 567 bacteria from root nodules of various summer legumes including *Glycine max* (Soybean), *Vigna radiata* (Mung bean), *Vigna unguiculata* (Cowpea), *Susbenia grandiflora* (Sesbania) and *Cymposistetra gonoloba* (Guar)

and found that only 9% of rhizobial isolates were able to produce orange red colouration on filter paper strips. Monika and Wati (2017) reported that out of 65 rhizobial isolates only six isolates were found positive for hydrogen cyanide production.

Some of the rhizobia are reported as phosphate solubilizers. In present study, P-solubilization index of rhizobial isolates varied

from 2.4 to 3.3 at 30°C (Table 1) while at 45°C the value ranged between 1.8 to 2.3 (Table 4). Maximum P-SI was observed in MRH 59 i.e. 3.3, 3.0, 2.8 and 2.3 at 30, 35, 40 and 45°C respectively followed by MRH 44 (3.1, 2.9, 2.7 and 1.9 P-SI at 30, 35, 40 and 45°C respectively). Monika and Wati (2017) reported that out of 65 only twelve rhizobial isolates showed phosphate solubilizing ability.

Table 1: Plant growth promoting multi traits of thermotolerant mungbean rhizobial isolates at 30°C

Rhizobial Isolate	IAA production (µg/ml)	Ammonia excretion (µg/ml)	Siderophore Production	HCN production	PSI
MRH4	23.15	3.65	+++	+++	2.4
MRH44	53.36	3.92	+++	+++	3.1
MRH46	55.76	4.34	+++	+++	2.9
MRH59	58.83	4.78	+++	+++	3.3

(+++), High production, (++) Moderate production, (+), Low production

Table 2: Plant growth promoting multi traits of thermotolerant mungbean rhizobial isolates at 35°C

Rhizobial Isolate	IAA production (µg/ml)	Ammonia excretion (µg/ml)	Siderophore Production	HCN production	PSI
MRH4	21.14	3.42	++	++	2.2
MRH44	50.85	3.69	++	++	2.9
MRH46	52.12	4.14	+++	+++	2.6
MRH59	56.58	4.50	+++	+++	3.0

Table 3: Plant growth promoting multi traits of thermotolerant mungbean rhizobial isolates at 40°C

Rhizobial Isolate	IAA production (µg/ml)	Ammonia excretion (µg/ml)	Siderophore Production	HCN production	PSI
MRH4	21.02	3.20	++	++	2.2
MRH44	45.63	3.61	++	++	2.7
MRH46	46.08	4.11	+++	+++	2.4
MRH59	54.77	4.39	+++	+++	2.8

Table 4: Plant growth promoting multitraits of thermotolerant mungbean rhizobial isolates at 45°C

Rhizobial Isolate	IAA production (µg/ml)	Ammonia excretion (µg/ml)	Siderophore Production	HCN production	PSI
MRH4	14.58	1.89	+	+	1.8
MRH44	37.15	2.15	+	+	1.9
MRH46	33.04	2.91	++	++	2.1
MRH59	39.86	3.32	++	++	2.3

CONCLUSION

The results of present study indicated that rhizobial isolate MRH 59 showed multiple plant growth promoting traits under at high temperature under *in vitro* conditions can be explored as inoculant for summer mungbean after authenticating under pot house and field conditions.

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