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Growth response of region specific *Rhizobium* strains isolated from *Arachis hypogea* and *Vigna radiata* to different environmental variables

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Six different strains isolated from *Vigna radiata* (IARI-1 UU-2, UU-4, UU-7, UU-10 and UU-13) and seven strains from *Arachis hypogea* (IARI-16, UU-17, UU-18, UU-19, UU-20, UU-21 and UU-22) were selected to assess their capability to tolerate environmental variables like pH, temperature, salinity (NaCl), iron (Fe), phosphate (K_2HPO_4), and nitrate ($NaNO_3$). The bacteria under investigation expressed noticeable ability to grow under these stress factors examined. The rhizobial isolates were compared to two reference rhizobial strains of specific host collected from IARI, India. *Rhizobium* UU-4 from *Vigna radiata* was found to be most tolerant species to high and low temperature, high salinity, relatively higher phosphate and higher nitrate concentrations but was sensitive to lower pH. UU-2 from the same host tolerated maximum to lower temperature, alkaline pH, phosphate deficiency and to higher concentration of iron but was very sensitive to a little raise in salinity. UU-10 was tolerant to lower pH, low nitrate, relatively higher phosphate, non-saline conditions and low iron concentration in the media. UU-13 grew well only in the presence of higher phosphate and low iron and was sensitive to lower temperature, acidic pH, phosphate deficiency and to the presence of nitrate in the media. UU-1 was most sensitive to higher temperature, alkaline pH, non-saline condition, higher concentration of phosphorous and nitrate, and was tolerant to higher level of salinity. UU-22 from *A. hypogea* was most tolerant to lower as well as higher temperatures, but sensitive to salinity and higher nitrate concentration. UU-21 was very sensitive to low temperature, salinity, presence of iron and also grew only when the nitrate concentration in the media was more than 50 µg/ml, but was tolerant to alkaline pH. UU-16 grew well in salinity free conditions, tolerant to low iron and also grew well in higher nitrate (up to 200 µg/ml) and phosphate deficiency, but was very sensitive when the concentrations of iron became higher. UU-17 preferred lower pH, lower temperature, moderate salinity, higher iron, phosphate deficiency and also grew comparatively well in presence of nitrate. UU-20 though was sensitive to lower pH and phosphate deficiency, was tolerant to higher salinity and also grew well in presence of nitrate. Based on the above results, *Rhizobium* strains UU-22 from *A. hypogea* and UU-13 from *V. radiata* may be effective for nodulation as well as yield of two leguminous crops.

Key words: Rhizobia, region specific, environmental stress, *Arachis hypogea*, *Vigna radiata.*

INTRODUCTION

Intensive agriculture, which is largely based on the use of nitrogen chemical fertilizer, is the opposite of sustainable agriculture based on repositioning of nitrogen used by plant growth through supply of organic residue and succession of legume crop (Popelka et al., 2004; Acharya et al., 1953). Besides legumes are important in such agriculture practices being a chief source of protein and also produced beneficial effects for soil fertility and conservation due to biological nitrogen fixation. Inoculation of efficient strains of rhizobia is important when a legume is introduced in a region. The efficiency of the legume-rhizobia symbiosis is affected by various environmental factors (Thies et al., 1995; Palmer and Young 2000; Yuhashi et al., 2000). *Rhizobium* is a number of genetically diverse and phylogenetically heterogenous groups of bacteria. Recently, it has been reported that rhizobial cultures are also used as growth promoters for non-leguminous plants (Hossain and Mårtensson, 2008). It has been well reported that *Rhizobium* inoculants are highly sensitive to slightest change in environmental conditions, especially in respect of soil reaction due to variation in pH, moisture conditions and variation in temperature (Michiels et al., 1994; Evans et al., 1993). Thus, *Rhizobium* strains from outside a particular agro-climatic zone often fail to achieve the desired result (Azad, 2004). Therefore, it becomes necessary to isolate and screen the native *Rhizobium* strains and testing the efficacy of *Rhizobium* biofertilizer with regards to their infective capability, production of effective nodules in the host and their contribution to growth and yield attributes of the inoculated crops (Saikia et al., 2006).

Environmental stresses play an important role in level of legume production. Among stress factors, salinity, pH, temperature, iron, nitrate and phosphate are most important in regulating the natural distribution of plant, is a very serious problem in many agricultural areas. Different stress limits legume growth, especially when the crop relies on symbiotically fixed N (Velagaleti et al., 1990). The isolation and characterization of rhizobial strains tolerant to stress condition may allow the prediction of their eventual behavior as a community in soils and in this way may lead to a better interaction with the plant for its later introduction into unfavorable soils. With the purpose of isolation of *Rhizobium* strains from different agro-climatic condition, in the present work, different *Rhizobium* strains were characterized and their growths under different environmental stress like at low and high pH, temperature, salinity (NaCl), iron (Fe), phosphate (K_2HPO_4) and nitrate ($NaNO_3$) conditions were studied.

MATERIALS AND METHODS

Isolation of *Rhizobium* strains

Thirty days old selected legume plants were uprooted, washed in distilled water and the well-formed, healthy and pinkish nodules on the tap roots were carefully cut out. The nodules were immersed in 95% (v/v) ethanol for 10 s, sterilized for 5 min in 0.1% acidified mercuric chloride ($HgCl_2$, 1g L^{-1}; conc. HCl, 5 ml L^{-1}) and washed six times with sterile distilled water to get rid of the chemical (Chen and Lee, 2001). Each nodule was crushed using a sterile glass rod in an aliquot of sterile distilled water. Serial dilutions of the suspension were made and an aliquot of appropriate dilution was plated on yeast-extract mannitol agar medium (YEMA) and incubated at 28±2°C for four to seven days (Bogino et al., 2008). Distinct colonies were picked up and transferred to agar slants for further purification. Confirmation of the *Rhizobia* was ascertained by streaking on YEMA medium supplemented with Congored (0.025%, w/v), bromothymol blue test, and EPS production (Hameed et al., 2004; Sethi and Adhikary, 2009). The *Rhizobia* stand out as white and translucent colonies (Subbarao, 1977). One week old rhizobial colonies kept on YEM agar media (1.5% agar) were used for preparation as inoculants. For this purpose, loop of the respective colonies were inoculated in sterile YEM medium in liquid broth. Strains were routinely maintained on YEMA slants at 4°C (Castro et al., 1997). In addition, two strains of *Rhizobium* of the respective hosts isolated and maintained at the Microbiology laboratory of IARI (Indian Agricultural Research Institute), New Delhi were used as negative control. Growth of all the 26 native and 2 IARI strains of *Rhizobium* was estimated at 12 h intervals up to stationary growth phase and growth was measured as the absorbance of the culture suspension at 600 nm.

Selection of strains

Totally, 13 strains of *Rhizobium* were isolated from *V. radiata* and *A. hypogea* cultivated southern region of Odisha state, India and maintained in culture in YEM media. In addition, two strains of *Rhizobium* of the respective hosts isolated and maintained at the Microbiology laboratory of IARI (Indian Agricultural Research Institute), New Delhi, India were used as negative control. Based on the higher growth rate, six *Rhizobium* isolates from *A. hypogea* and five isolates from *V. radiata* were chosen and their growth pattern under various environmental variables was examined in culture. Strain number UU stands for Utkal University and IARI-Indian Agricultural Research Institute.

Growth response of *Rhizobium* species from *V. radiata* and *A. hypogea* under various environmental variables

Based on the higher growth rate, seven *Rhizobium* isolates from *A. hypogea* and six isolates from *V. radiata* were chosen and their growth pattern under various environmental variables was examined in triplicate in culture. These were: pH of the medium ranging from 5-10, at different temperatures (4, 25, 28, 30, 35 and 45°C), salinity ranging from 0 to 1 M and in presence and absence of various concentration of nitrate ($NaNO_3$), phosphate (K_2HPO_4) and iron (Fe- citrate). Growth response of the selected *Rhizobium* isolates at various pH levels (from 5 to 10), temperature gradients (4-45°C), salinity (0 to 1 M), nitrate (0 to 1 mg/ml), phosphate

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(0 to 20 mg/ml) and iron (0 to 300 µg/ml) was examined. The different concentrations of the treatments were prepared in YEMA medium and the organisms were grown by inoculating uniform amount of culture suspension into the experimental tubes. Corning hard glass test tubes of the size (18 x 200 mm) plugged with non-absorbent cotton wool was used and totally 10 ml of suspension including the inoculums culture were incubated in an incubator for up to 72 h. Triplicates were set for each set of experiments and mean of 3 closely concordant determinations were calculated and presented in text.

RESULTS

Growth pattern of six *Rhizobium* strains from *Vigna radiata* subjected to various temperature for example, 4, 25, 28, 30, 35 and 45°C, pH (5, 6, 7, 7.8, 9 and 10), NaCl (ranging from nil to 1 M), sodium nitrate (nil to 1 mg/ml), phosphate (nil to 20 mg/ml) and iron as citrate (nil to 300 µg/ml) was examined. Unless otherwise stated, the cultures were maintained at 28°C and pH 7.8 throughout the growth period (Figure 1A to F). Maximum growth of all the strains was obtained at 30°C with little change in temperature range of 25 to 35°C. Growth of IARI-1, UU-2 and UU-7 were considerably affected at 45°C in comparison to other strains, however, UU-4, UU-10 and UU-13 showed almost similar growth at the temperature range of 25 to 30°C with little less at lower temperature (Figure 1). Similarly, all these isolates grew well at pH 7.8 and increase or decrease of pH of the culture to acidic or alkaline range showed detrimental effect on the growth of these isolates; although at 4 and 45°C, and pH 5 and 10, respectively of the culture did not support good growth of the *Rhizobium* isolates. UU-2, UU-7 and UU-13 were quite tolerant to pH from 7 to 9 (Figure 1). All these isolates grew well in presence of 0.025 M NaCl (control). Upon increase of the NaCl concentration up to 0.1 M in the media except for IARI-1 and UU-10, the growth of all other strains decreased in presence of higher concentration of NaCl. Growth of all the isolates though were affected in absence of NaCl, none of them could tolerate up to 1 M NaCl, and in many, for example, UU-10 and UU-13, even growth was drastically reduced in presence of 0.5 M NaCl (Figure 1). Growth of all the isolates except IARI-1 and UU-4 was progressively enhanced in the presence of up to 0.05 mg/ml of $NaNO_3$ in the medium except in the case of UU-10; where, more than 0.02 mg/ml of nitrate did not support higher growth.

To the contrary in UU-2 and UU-7, highest growth was obtained even in presence of 0.05 mg/ml of nitrate. Further increase in the growth of all these strains was decreased and growth was static in UU-7 and UU-10 in the presence of 1 mg/ml of nitrate (Figure 1). When phosphate was not supplemented in media, growth of almost all the strains was decreased up to 45%. Similarly, with increase of the phosphate concentration in the media, growth was affected than that of control culture and the adverse effect was proportionate with increase of phosphate up to 10 mg/ml. With further increase, growth of all the isolates were severely affected (Figure 1).

Since Odisha soil is rich in iron, tolerance of the *Rhizobium* isolates from *V. radiata* to increase in iron concentration is of immense importance. The results show that UU-2, UU-4 and UU-7 tolerated and grew higher in presence of up to 10 µg/ml of iron citrate. With further increase in iron concentration, growth of all strains was adversely affected. The adverse effect of iron was comparatively less pronounced in UU-10, UU-2, UU-4 and UU-7 up to 200 µg of iron/ml. However, with further increase of iron up to 300 µg/ml, the growth of all the strains were decreased up to 80 to 90% (Figure 1).

Quite different from the growth response of *Rhizobium* from *V. radiata*, the rhizobia from *A. hypogea* showed less tolerance to change in the environmental stresses as above, but grew well in presence of higher concentrations of the phosphate in the medium. Though optimum growth of these rhizobia from *A. hypogea* was seen at 28°C with little higher temperature to 30°C, growth of UU-17, UU-18, UU-19, UU-21 and UU-22 were adversely affected by 6 to 14% and less. With further increase to 35°C, UU-16 was more sensitive and decreased the growth by 40% and all the other six strains growth decreased from 21 to 33%. Almost similar decrease in growth from 12-37% was seen at 25°C. With further decrease of temperature to 4°C or increase up to 45°C, the growth of all these strains decreased from about 56 to 68% (Figure 2). Similarly, with the increase in pH of the culture from 7.8 to 8, growth of all the IARI-16, UU-18, UU-20 and UU-22 decreased from 4 to 6%, and the decrease was more pronounced with further increase of pH up to 10 and also with decrease of the pH in the order of 7, 6 and 5 proportionately; at pH 5, growth of all these strains was inhibited by 47 to 81% and at pH 10, decrease of the growth was in the range of 30 to 53%. The results show that all the rhizobia from *A. hypogea* were more tolerant to alkaline pH than acidic conditions (Figure 2).

All the seven rhizobial strains from *A. hypogea* were sensitive to slight change in the NaCl concentration of the medium. In the absence of NaCl, growth was inhibited by 28 to 47%. With increase of the NaCl concentration in the medium, growth of all the strains was progressively decreased with proportionate increase in concentration of the salt, and at 1 M, growth of all the organisms was inhibited by 64 to 81% (Figure 2) showing that unlike rhizobia from *V. radiata,* rhizobia from *A. hypogea* were unable to tolerate in the saline conditions of the soils. The organisms grew well in media in the absence of nitrate. Growth of IARI-16 and UU-22 remained unchanged in presence of up to 0.02 mg/ml of nitrate, however, in all other strains, growth was decreased by 6 to 8% in presence of the low concentration of nitrate (Figure 2C). With the increase of NaCl in a medium from 0.5 up to 1 mg/ml, growth of all these strains decreased proportionately to the increase of the nitrate concentration and at 1 mg/ml in the media; growth of these strains was decreased from 66 to 82%. All the *Rhizobium* strains from *A. hypogea* were slightly sensitive to increase in

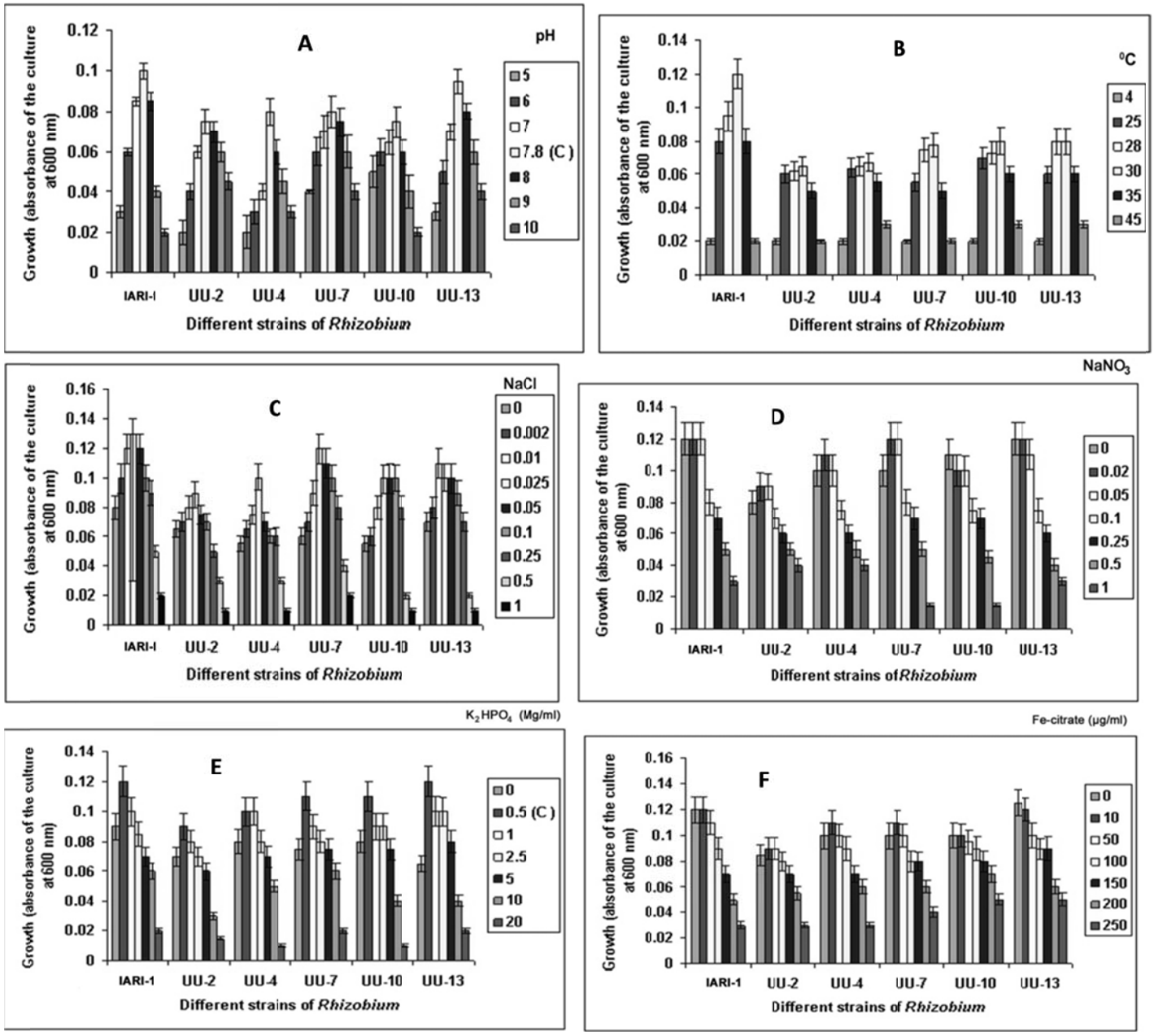

Figure 1. A - F. Growth of different strains of *Rhizobium* isolated from *Vigna radiata* from different temperature (°C), pH, salinity (NaCl), nitrate ($NaNO_3$), iron (Fe-citrate) and phosphate (K_2PO_4). Cultures were incubated for 120 h at 28±2°C. Initial inoculum of the culture suspension at 600 nm was 0.02. IARI-1, IARI culture strain Delhi; UU-2, Gobindapur; UU-4, Maniakati-2; UU-7, Paduraisuni; UU-10, Lathipada; UU-13, Asurabandha.

concentration of iron at 250 µg/ml of iron citrate. Growth of UU-19, UU-20, UU-21 and UU-22 was completely ceased at this concentration though IARI-16 and UU-17 growth was inhibited from 51-66% at the same iron concentration.

However, at low concentration of iron (10 µg/ml), growth of IARI-16, UU-21 and UU-22 almost remained static. In all other strains especially in UU-18, growth was inhibited by 21% even in presence of 10 µg/ml of iron citrate (Figure 2E). With increase in concentration of iron, growth of all the strains decreased in presence of 150-200 µg/ml iron, which is the usual iron concentration of most red soils of Odisha (Sahu et al., 1996). Growth of all these strains was inhibited by 33-53% suggesting that iron rich soils of the region are not conducive for the *Rhizobium* of *Arachis hypogea*. It was important to find that in phosphorous deficient media, growth of all these strains of rhizobia from *Arachis hypogea* was decreased

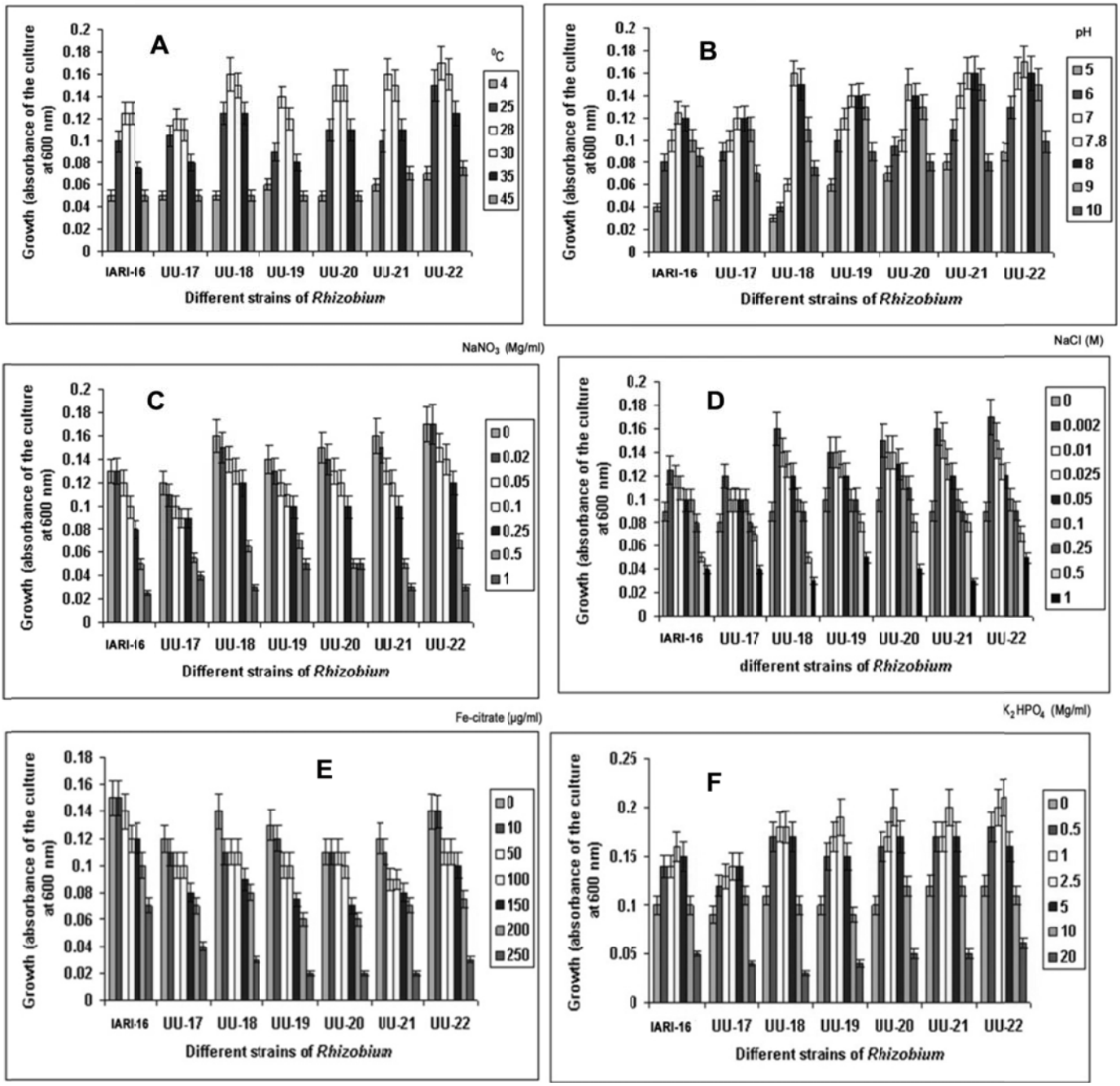

Figure 2. A - F. Growth of different strains of *Rhizobium* isolated from *Arachis hypogea* from different temperature (°C), pH, salinity (NaCl), nitrate ($NaNO_3$), iron (Fe-citrate) and phosphate (K_2PO_4). Cultures were incubated for 120 h at 28±2°C. Initial inoculum of the culture suspension at 600 nm was 0.02. IARI-16, IARI culture strain Delhi; UU-17, Maniakati-4; UU-18, Maniakati-5; UU-19, Amrutulu; UU-20, Buguda; UU-21, Khilabadi; UU-22, Surada-1.

by 28-33%.

However, growth was progressively increased with increase of phosphorous as K_2HPO_4 in the media from 0.5 to 2.5 mg/ml; the increase was more prominent in UU-19, which was up to 26% followed by the strain IARI-16, UU-17, UU-18, UU-20 and UU-22. Growth of most strains except UU-22 either remained unchanged or increased up to 12% in the presence of 5 mg/ml phosphate, but with further increase of the nutrient, growth was adversely affected; the decrease of the growth was up to 64 to 82% in these strains in the presence of 20 mg/ml of K_2HPO_4 (Figure 2F).

Comparative analysis of the growth pattern of severa strains of *Rhizobium* from *V. radiata* and *A. hypogea* to

Table 1. Comparative study of growth response of several strains of *Rhizobium* species isolated from *Vignaradiata* to low and high pH, temperature, salinity, iron, phosphate and nitrate.

Parameter		Strain
Temperature	Low (25°C)	UU-7< UU-13< IARI-1< UU-10< UU-2< UU-4
	High (45°C)	IARI-1< UU-7< UU-2< UU-13< UU-10< UU-4
pH	Low pH (6)	UU-4< UU-13< UU-2< IARI-1< UU-7< UU-10
	High pH (9)	IARI-1< UU-10< UU-4< UU-13< UU-2< UU-7
Salinity	Zero	IARI-1< UU-4< UU-7< UU-13< UU-10< UU-2
	High (0.25 M)	UU-10< UU-2< UU-7< UU-13< IARI-1< UU-4
Nitrate	Low (50 µg/Ml)	UU-13< UU-7< UU-4< IARI-1< UU-2< UU-10
	High (200 µg/Ml)	UU-13< IARI-1< UU-7< UU-10< UU-4< UU-2
Phosphate	Zero	UU-13< UU-7< UU-10< IARI-1< UU-2< UU-4
	High (2.5 Mg/Ml)	IARI-1< UU-7< UU-2< UU-4< UU-10< UU-13
Iron	Low (0.05 Mg/Ml)	UU-7< UU-2< IARI-1< UU-4< UU-10< UU-13
	High (0.25 Mg/Ml)	UU-13< IARI-1< UU-4< UU-10< UU-7< UU-2

Table 2. Comparative study of growth response of several strains of *Rhizobium* species isolated from *Arachis hypogea* to low and high pH, temperature, salinity, iron, phosphate and nitrate.

Parameter		Strain
Temperature	Low (25°C)	UU-21< UU-19< UU-20<UU-18< IARI-16< UU-17< UU-22
	High (45°C)	UU-18< UU-20< UU-19< IARI-16< UU-17< UU-21< UU-22
pH	Low pH (6)	UU-20< IARI -16< UU-21< UU-19< UU-17< UU-22< UU-18
	High pH (9)	UU-18< IARI -16< UU-20< UU-22< UU-17< UU-19< UU-21
Salinity	Zero	UU-22< UU21< UU-18< UU-20< UU-17< UU-19< IARI -16
	High (0.25 M)	UU-22< UU-21< UU-18< IARI -16< UU-17< UU-19< UU-20
Nitrate	Low (50 µg/ml)	UU-21< UU-19< UU-22< UU-18< UU-17< UU-20< IARI -16
	High (200 µg/ml)	UU-19< UU-22< UU-20< UU-18< UU-21< UU-17< IARI -16
Phosphate	Zero	UU-20< UU22< UU-19< UU-21< UU-18< IARI -16< UU-17
	High (2.5 mg/ml)	UU-19< UU-20< UU-21< UU-22< IARI -16< UU-17< UU-18
Iron	Low (0.05 mg/ml)	UU-21< UU-17< UU-19< UU-20< UU-18< UU-22< IARI -16
	High (0.25 mg/ml)	IARI -16< UU-21< UU-20< UU-22< UU-19< UU-18< UU-17

lower and higher pH (6 and 9), temperature (25 and 45°C), salinity (0 and 0.25 M NaCl), iron (0.05 and 0.25 mg/ml of Fe-citrate), phosphate (0 and 2.5 mg/ml) and nitrate (50 and 200 µg/ml) was analyzed and given in Tables 1 and 2. It was found that considerable variation exists between these organisms on the basis of their resistance to several of these environmental variables. UU-4 from *V. radiata* was found to be most tolerant species to high and low temperature, high salinity, phosphate deficiency, relatively higher phosphate and higher nitrate concentrations, but was sensitive to lower pH.

Next to this, UU-2 from the same host tolerated maximum to lower temperature, alkaline pH, phosphate deficiency and to higher concentration of iron, but was very sensitive to growth at little increase in salinity. UU-10 was tolerant to lower pH, low nitrate, relatively higher phosphate in non-saline conditions and in low iron concentration in the media. UU-13 grew well only in the presence

of higher phosphate and low iron, and was sensitive to lower temperature, acidic pH, high iron concentration, phosphate deficiency and to presence of nitrate in the media. IARI-1 was most sensitive to higher temperature, alkaline pH, non-saline condition, higher concentration of phosphorous and nitrate, and was tolerant to higher level of salinity (NaCl).

DISCUSSION

These results show that the same organism did not grow well or were tolerant to either all the stresses or even low and high value of a particular environmental stress that might be occurring in the crop fields. Physico-chemical characteristics of different agro-climatic regions of Odisha showed wide variation in the pH, iron, phosphate, nitrate, conductivity as well as salinity of these soils (Sahu et al., 1996). Hence, it is essential to select a particular strain from the desired crop suitable to its capability to grow under these variables of the crop fields of a particular region so that the inoculated strain can establish and perform leading to higher productivity. Based on the results above on the tolerance of six strains of *Rhizobium* from *V. radiata* and seven strains from *A. hypogea,* three strains each, IARI-1, UU-4 and UU-10 from the former and UU-20, UU-21 and UU-22 from the later host species were selected and changes in their protein profile in response to various environmental stresses was analyzed.

Maximum growth of *Rhizobium* isolated from *V. radiata* and *A. hypogea* was obtained at 28°C and with little increase or decrease of temperature, they had a significant effect on their growth. Maximum soil temperature in the tropics usually exceeds 45°C at 5 cm and 50°C in 1 cm depth (Lal, 1993), and can limit nodulation in relation to rhizobial growth. Upper limit ranges between 32 and 47°C, although tolerance varies among species and strains because high temperature decreases rhizobial survival and establishment in tropical soils.

Hence, repeated and higher rates of inoculation may frequently be needed. The alternative is inoculated strains capable of surviving at the higher temperature of tropics so as to make the inoculation successful. There have been number of investigations on the effect of temperature on infection process of temperate species of *Rhizobium* in environmental growth chamber. The results show that below 10°C, root hair infection by *Rhizobium* is retarded whereas at 24°C and above; the rate of infection is enhanced. However, these results are dependent on variation between *Rhizobium* strains and host cultivars.

The same is true in tropical climatic regime with a higher temperature limit. Rhizobia are known to survive in stored dried soil for several years (Sen and Sen, 1958) and could tolerate 45°C and produce nodules on roots of *Vigna mungo*. Further testing under field condition revealed that this heat tolerant strain of *Rhizobium* significantly increased grain yield of *V. mungo* (Subbarao, 1982).

Rhizobium from both crops grew well at near neutral pH and with variation of the pH to acidic or alkaline pH, their growth were affected though there were minor deviations among the strains to tolerate higher and lower pH levels. The optimum pH for rhizobial growth has been found between pH 6 to pH 7 (Jordan, 1984) with relatively few rhizobia growth in acidic pH (Graham et al., 1994). Intrinsic tolerance cannot be predicted from the pH at the site of isolation because when fast growing rhizobial strains were isolated from nodules that have been inoculated with soil from certain sites where the pH ranges from 3 to 5, only 37% were able to grow in buffered medium at pH 4 and 60% grew at pH 9.5 (Hungria and Vargas, 1996).

A large proportion of tropical soils have developed from old geological formation. This combined with climatic conditions has resulted in highly weathered soils containing predominantly low activity clays. These are usually acidic and infertile, and frequently contain toxic chemicals. Such acid soil conditions pose problems for plants, the bacteria and the symbiosis (Giller and Wilson 1993). The microsymbiont is usually more sensitive to pH. Some rhizobial species can tolerate acidity better than others, however, similar results that the tolerance may vary among strains within a species has been reported earlier (Brockwell et al., 1995, Hungria et al., 1997)

Different species of rhizobia withstand different levels of NaCl, which was invariably higher than the host plant (Subbarao, 1974). Further degree of salinity/alkalinity conducive for good nodulation was different from the limits of tolerance of *Rhizobium* and the host to the salt. Of these, growth responses of several strains of *Rhizobium* from *V. radiata* and *A. hypogea* to different concentration of NaCl ranging from 0.002 to 1 M, showed wide variation in the capabilities of these strains to tolerate the salt.

There are reports that salt tolerant strains significantly enhance their capacity to oxidize carbon sources by increasing growth rate and EPS production that involve in adhesion resulting in a greater adapting capacity to colonize on favorable saline environment (Barboza et al., 2000). Lippi et al. (2000) has studied the effect of salinity on growth, starvation, survival and recovery from salt stress of a *Rhizobium* isolated from nodules of *Acacia*. The results show that survival capacity of starved cultures depended on previous growth condition and culturability subjected to double stress starvation and salinity was reduced considerably. All the starved cultures were capable of regrowth when nutrients became available thus showing
that the strain can withstand long periods of nutrient deprivation in soil while maintaining the capacity for an active metabolism and a potential infectiousness to the host.

All the *Rhizobium* species isolated from *V. radiata* and

A. hypogea grew well in presence of up to a tolerant limit of $NaNo_3$, though *Rhizobium* from *V. radiata* were invariably more tolerant to nitrate than those isolated from *A. hypogea.* There are reports that legume can use nitrogenous fertilizer and grow well but application of such fertilizers, especially at higher doses inhibit nodule number, efficiency of fixation, bacteroids and membrane envelope formation showing that it diminishes all attributes of symbiosis (Subbarao, 1974). Similarly, another soil nutrient phosphate though is essential for growth of all the rhizobia, the ones from *V. radiate* required comparatively less phosphorous than from the other host to grow. Earlier reports showed that application of phosphate to leguminous crops enhances the number of nodules, the nitrogen content and growth of plants (Vyas and Desai, 1953). Acharya et al. (1953) have shown that rotation of crops and phosphate manure enhances soil nutrient content. The results of the present investigation together with the earlier reports show that these two nutrients, nitrate and phosphate are essential at certain concentration for the growth of rhizobia in the soil but the critical concentration as per the requirement varies from species to species.

Although iron is abundant in soil (1 to 6%) and it ranks 4th among all elements on surface of earth, it is often unavailable to the microbes and plants because of its solubility, which is dependent on pH. Under aerobic soil conditions, most iron exists in the insoluble ferric form (Dudeja et al., 1997). It is a component of the cell and its deficiency causes growth inhibition and can also change the cell morphology. To meet the requirement of iron, the organisms evolve a specific high affinity mechanism and when the medium and the soil is low in soluble iron, this mechanism becomes operative, and this happens with involvement of siderophores, which are low molecular weight iron chelators (Dudeja et al., 1997). Iron plays special role in root nodules for the symbiotic nitrogen fixation as this is required for leghaemoglobin, nitrogenase and cytochrome synthesis within the bacteroids in the nodules. Research have shown that presence of active nodules indicate iron deficient stress response in soybean (Dudeja et al., 1997). Odisha soils are rich with iron, which varies from 8 to 376 ppm (Sahu et al., 1990). The locations where the field experiments for the present work were conducted are rich with iron exceeding 100 µg/g soils. Growth response of these strains to various iron concentrations is a critical factor for their establishment after inoculation to make the biofertilizer programme successful. Hence, selection of iron tolerance strains and those grown at comparatively higher iron concentrations were specially taken care for selection of strains for further experiments.

Conclusion

The above experimental results show that *Rhizobium* from both the crops *A. hypogea* and *V. radiata* in response to the same stress was quite different. This shows that there may exists a genetic variability among the rhizobial strains from the same host and also from different host plant to cope with the stress factors prevailing in a specific location. The results clearly demonstrated that rhizobium isolated from the local environments are more tolerant to these environmental stresses than strains collected from IARI, New Delhi, India which belongs to different agro-climatic condition. Hence, it can be concluded that the host as well as the region specific rhizobium isolates is more important for making a biofertilizer programme successful.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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