Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883**
Sch. Acad. J. Biosci., 2013; 1(5):179-182

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Research Article

A comparative study of Effect of Rhizobacteria onto seedling vigour of groundnut in *in-vitro* condition isolated from Kuttch region

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Abstract: Some high salt tolerant Plant Growth Promoting bacteria(BM6, AMAAS57, AMAAS357, R29 and R5) were isolated from Gujarat kuttch region. Their PGP characters were characterized at lab by different biochemical test. To study their ability to promote growth of plant in vitro bioassay of the selected isolates was done. The bioassay was done with groundnut seedling under 2 ds/m, 4ds/m and 6ds/m salt stressed condition. Different growth parameters of groundnut seedling were measured after 7 days growth. Statistical analysis of the experimental data was done following the SPSS package. The results revealed that primary root length was improved significantly over uninoculated control when the seedlings were inoculated with Enterobacter sp. R29. However, treatment of seedlings with other rhizobacteria did not influence the primary root growth except with Pseudomonas aeruginosa AMAAS57 wherein the primary root growth was reduced significantly. The secondary root length was improved significantly when inoculated with Pseudomonas sp. AMAAS357, Pseudomonas aeruginosa BM6 and Enterobacter sp. R29 without salinity stress. However, the number of secondary root was improved significantly only when seedlings were treated with Enterobacter sp. R29 and fresh weight of the seedlings was improved significantly only when inoculated with Pseudomonas sp. AMAAS357.
Keywords: Plant Growth Promoting bacteria, salinity, rhizobacteria, seedling.

INTRODUCTION

Salinity is a major environmental factor that causes degradation of the physico-chemical properties of soil resulting in major impacts on crop productivity. Globally, approximately one billion ha of land (7% of total land area) are affected by soil salinity throughout the arid and semi-arid regions of the world. This is estimated to have a negative impact on one-third of the world's food production system [5]. This is clearly an enormous problem, and of great concern. Between 30-40% of the world's irrigated lands are prone to salinity [3]. In India, around 25000-30000 ha of cultivated land is coming under salinity each year. The problem is likely to aggravate further due to erratic rainfall, climate change, and ingression of sea water and accumulation of salts from various natural and anthropogenic sources. Salt in the environment can result from natural weathering of geological formations or from anthropogenic activities.

To minimize the effects of soil salinization, much efforts have been made for finding economical and effective methods to re-establish vegetation in salt-impacted soils [1,8]. Some methods that have been used for removal of salt in soil include disposal of surface layers, use of electro-kinetic extraction, soil washing with clean water, or soil mixing with organic materials to improve soil structure [1,8]. Unfortunately, these techniques are often impractical and costly as well as having other environmental drawbacks such as inappropriate disposal of the contaminants.

To overcome the salt stress on plants, it has been found that groups of free-living rhizobacteria called plant growth promoting rhizobacteria (PGPR) that contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase which can be applied to plant seeds to lower the plant stress hormone, ethylene, and promote plant growth under salt stress. Plant growth-promoting rhizobacteria (PGPR) are a group of free-living saprophytic bacterial micro-organisms that live in the plant rhizosphere and aggressively colonize the root system, and have been studied as plant growth promoters for increasing agricultural production and as biocontrol agents against plant diseases [2]. More specifically, the soil-borne fluorescent pseudomonads have received particular attention throughout the global science because of their catabolic versatility, excellent root colonizing ability and their capacity to produce a wide range of enzymes and metabolites that favour the plant withstand under varied biotic and abiotic stress conditions [4,6,9].

MATERIALS AND METHODS:

Bioassay of the selected isolates was done for studying their ability to promote groundnut seedling growth under saline condition *in vitro*.

Groundnut seeds were surface sterilized using $HgCl_2$ solution and geminated in Petridishes containing 1% agar. Then the germinated seeds were coated with isolated pseudomonad and rhizobia culture. Coated germinated seeds were inoculated in Petriplates containing 1% agar amended with different salt concentrations i.e 2 dS/m, 4 dS/m, 6dS/m and a control. After 7 days following observations were taken-

- Shoot Length
- Primary Root Length
- Secondary Root Length
- Secondary Root Number
- Biomass

Statistical analysis

Statistical analysis of the experimental data was done following the SPSS package. All results were subjected to the least significant difference (LSD) test between means. The correlation co-efficient between a pair of means of related traits was determined. Standard deviation was determined following the standard procedures whatever required. The population densities of the isolates were estimated after log transformation of individual estimation

RESULT

Effect of rhizobacteria onto seedling vigour of groundnut in *in vitro* condition:

The plant growth-promoting rhizobacterial isolates were tested in vitro in Petridish bioassay with germinating seedlings to ascertain their role in enhancing the seedling vigour. The experiments were conducted with or without imposing salinity with cultivar GG2. It was found that primary root length was improved significantly over uninoculated control when the seedlings were inoculated with Enterobacter sp. R29 (Table 1). However, treatment of seedlings with other rhizobacteria did not influence the primary root growth except with Pseudomonas aeruginosa AMAAS57 wherein the primary root growth was reduced significantly (Table 1). The secondary root length was improved significantly when inoculated with Pseudomonas sp. AMAAS357, Pseudomonas aeruginosa BM6 and Enterobacter sp. R29 without salinity stress. However, the number of secondary root was improved significantly only when seedlings were treated with Enterobacter sp. R29 and fresh weight of the seedlings was improved significantly only when inoculated with Pseudomonas sp. AMAAS357 (Table 1). The effect on shoot length was non-significant.

Table 1: Evaluation of the effect of rhizobacteria on the in vitro seedling vigour of groundnut, cultivar GG2 (data mean of three replications) in Petri dishes

Treatments	Shoot Length (cm/p)	Primary Root Length (cm/p)	Secondary Root Length (cm/p)	Secondary Root Number /p	Fresh Weight (g/seedling)
Control	3.39	3.01	2.39	16.76	1.65
AMAAS57	2.62	1.46	1.54	7.62	1.43
AMAAS357	3.80	4.36	5.23	17.86	2.04
BM6	2.83	4.80	4.06	14.93	1.82
R29	3.31	5.19	3.99	22.97	1.77
R5	3.63	4.06	2.02	18.33	1.76
LSD (0.05)	**NS**	**1.87**	**0.98**	**6.14**	**0.24**

Evaluation of the effect of salinity on seedling vigour was also tested and was found to influence secondary root length and fresh weight of the seedlings significantly without any inoculation of rhizobacteria (Table 2). But effect on shoot and primary root length and secondary root number did not affect significantly among the salinity treatments (0, 2, 4 and 6 ds/m) (Table 2). Therafter, a separate experiment was conducted to evaluate the effect of both salinity and inoculation of salinity tolerant plant growth-promoting rhizobacteria on the seedling vigour and other growth parameters in vitro.

Evaluation of the rhizobacteria in alleviating the salinity stress in in vitro conditions indicated that there was non-significant impact of inoculation of PGPR on the primary and secondary root length of the seedlings of groundnut cultivar GG2 (Table 3) but there was improvements in the length of shoot and primary root when inoculated with Enterobacter sp. R29 (Table 3). Application of the PGPR isolates like Pseudomonas sp. AMAAS357 and Enterobacter sp. R29 significantly improved the secondary root length at 4 dSm-1 and 2 dSm-1, respectively as compared to uninoculated treatment and without application of NaCl (Table 3). At ECe 6 dSm-1, germination percentage was increase with the application of Enterobacter sp. R29 as

compared to uninoculated control. The number of secondary roots also increased appreciably when inoculated with Enterobacter sp. R29 upto 4 ECe and Pantoea dispersa R5 at 4 and 6 ECe (Table 3). The effect on fresh weight of the seedlings was non-significant.

Table 2: Evaluation of the effect of salinity on the in vitro seedling vigour of groundnut, cultivar GG2 (data mean of three replications)

Salinity level	Shoot length	Primary Root Length	Secondary Root Length (cm/p)	Secondary Root Number/p	Fresh weight (g/seedling)
0	3.62	4.47	3.86	16.85	1.80
2 dSm^{-1}	3.33	3.41	3.39	15.54	1.77
4 dSm^{-1}	3.25	4.18	3.50	18.23	1.82
6 dSm^{-1}	2.83	3.19	2.06	14.70	1.58
LSD	**NS**	**NS**	**0.80**	**NS**	**0.19**

Table 3 : Evaluation of the effect of application of rhizobacteria on the *in vitro* seedling vigour of groundnut, cultivar GG2 at different level of salinity (data mean of three replications)

Level of salinity and treatments	Shoot length (cm/p)	Primary Root Length (cm/p)	Secondary Root Length (cm/p)	Secondary Root Number/p	Fresh weight (g/seedling)
0 ECe + control	3.64	6.05	5.61	19.22	1.86
2 ECe + Control	3.81	6.03	2.76	14.13	1.88
4 ECe + Control	3.22	4.03	4.30	17.89	1.92
6 ECe + Control	2.80	3.10	3.56	15.78	1.63
0 ECe + R29	4.25	6.95	4.62	28.22	1.92
2 ECe + R29	3.65	3.28	7.67*	25.05	1.97
4 ECe + R29	2.68	7.32	6.42	25.89	1.70
6 ECe + R29	2.68	3.21	3.44	10.72	1.50
0 ECe + R5	4.04	2.61	1.56	10.55	1.63
2 ECe + R5	3.16	3.93	1.61	13.89	1.58
4 ECe + R5	4.47	5.98	2.48	25.55	2.04
6 ECe + R5	2.83	3.73	2.42	23.33	1.81
0 ECe + AMAAS57	3.29	2.16	2.09	9.00	1.53
2 ECe + AMAAS57	2.90	1.53	1.40	6.48	1.52
4 ECe + AMAAS57	2.25	1.55	1.96	10.33	1.51
6 ECe + AMAAS57	2.03	0.60	0.72	4.67	1.18
0 ECe + AMAAS357	3.62	5.44	6.30	18.58	2.16
2 ECe + AMAAS357	3.89	2.69	4.41	14.89	1.94
4 ECe + AMAAS357	4.22	4.48	8.39*	19.78	2.30
6 ECe + AMAAS357	3.42	4.83	1.81	18.22	1.76
0 ECe + BM6	2.88	3.64	2.97	15.58	1.72
2 ECe + BM6	2.58	3.02	2.71	18.78	1.83
4 ECe + BM6	2.67	1.73	2.46	9.94	1.48
6 ECe + BM6	3.19	3.64	1.43	15.45	1.58
CD (0.05)	**NS**	**NS**	**1.96**	**NS**	**NS**

DISCUSSION

Evaluation of the effect of rhizobacteria onto seedling vigour of groundnut in *in vitro* conditions:

Presence of excess amount of salt in the growth media adversely affects growth and development of plants. Process such as germination, seedling growth and vigour, vegetative and other stages of plant growth are affected due to the stress [7]. Attempts have been made to understand whether salinity stress tolerant rhizobacteria can alleviate salinity stress in groundnut. In the present studies, all the five isolates were tested in vitro in Petridish bioassay with germinating seedlings to ascertain their role in enhancing the seedling vigour with cultivar GG2 in stressed conditions.

The results revealed that primary root length was improved significantly over uninoculated control when the seedlings were inoculated with Enterobacter sp. R29. However, treatment of seedlings with other rhizobacteria did not influence the primary root growth except with Pseudomonas aeruginosa AMAAS57 wherein the primary root growth was reduced significantly. The secondary root length was improved significantly when inoculated with Pseudomonas sp. AMAAS357, Pseudomonas aeruginosa BM6 and Enterobacter sp. R29 without salinity stress. However, the number of secondary root was improved significantly only when seedlings were treated with Enterobacter sp. R29 and fresh weight of the seedlings was improved significantly only when inoculated with Pseudomonas sp. AMAAS357.

CONCLUSION

Evaluation of the rhizobacteria in alleviating the salinity stress in in vitro conditions revealed that there was non-significant impact of inoculation of PGPR on the primary and secondary root length of the seedlings of groundnut cultivar GG2 but there was improvements in the length of shoot and primary root when inoculated with Enterobacter sp. R29. Application of the PGPR isolates like Pseudomonas sp. AMAAS357 and Enterobacter sp. R29 significantly improved the secondary root length at 4 ds/m and 2 ds/m, respectively as compared to uninoculated treatment and without application of NaCl. At ECe of 6 ds/m, germination percentage was increase with the application of Enterobacter sp. R29 as compared to uninoculated control. The number of secondary roots also increased appreciably when inoculated with Enterobacter sp. R29 upto 4 ECe and Pantoea dispersa R5 at 4 and 6 ECe though the effect on fresh weight of the seedlings was non-significant.

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