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ALLEVIATION OF SALINITY STRESS IN GROUNDNUT BY APPLICATION OF PGPR

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Abstract-*Two high salt tolerant Plant Growth Promoting bacteria(BM6, AMAAS57) 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 under salinity, pot trial was conducted using groundnut. Different physiological parameters (carbohydrate, phenol and free amino acid, SLA, RWC) were studied. Two pseudomonas culture having plant growth promoting tratits like production of IAA, HCN, ammonia, phosphate solubilisation, antifungal activity and tolerant to salinity (10% NaCl). These cultures were identified by 16s rRNA sequencing viz. Pseudomonas aeruginosa AMAAS57 and Pseudomonas aeruginosa BM6. Application of Pseudomonas aeruginosa AMAAS57 increased the production of phenol and free amino acids with soil salinity of 2 ds/m but thereafter decreased gradually with increasing salinity the decrease as compared to control. application of Pseudomonas aeruginosa AMAAS57 lowered the level of RWC% with increase in salinity. Application of Pseudomonas aeruginosa AMAAS57 and Pseudomonas fluorescens BM6 reduced the electrolyte leakage.*

Key words: Salinity alleviation, RWC%; Ds/m; SLA; RWC%; electrolytic leakage; carbohydrate, phenol and free amino acid content**.**

1. INTRODUCTION:

Salinity is a major environmental constraint to crop productivity throughout the arid and semi-arid regions of the world. In India, around 25000-30000 ha of cultivated land is coming under salinity each year affecting crop productivity and thus, management of salinity is of urgent need to sustain productivity.

Soil salinity has been reported to limit productivity of crops by impairing the root growth, nutrient uptake and by affecting the metabolic processes of the plant. Due to impairment of root growth and development, the active rhizosphere zone gets reduced and thereby, uptake of nutrients from the 'limited pool of available nutrients' becomes difficult. Besides, salinity also affects the nodulation and nitrogen fixation processes in leguminous plants [17], [8]. Salinity stress also decreases photosynthetic capacity of plant due to osmotic stress and partial closure of stomata [2]. Salinization/alkalization is known to limit nodulation and nitrogen fixation. High salt tolerance aids in tolerance to high pH and temperature. Several Rhizobium species have been reported from salt stressed soil in India and around the world.

The synthesis and activity of nitrogenases in A. brasilense is inhibited by salinity stress [19]. The accumulation of compatible solutes such as glutamate, proline, glycine, betaine and trehalose in response to salinity/osmolarity in Azospirillum sp. associated with rice cultivated along the coastline of Tamil Nadu has been reported.

In addition to the use of traditional breeding and plant genetic transformations, the use of plant growth promoting micro organisms may prove useful in developing strategies to facilitate plant growth in saline soils [12].

Plant growth promoting rhizobacteria (PGPR) and fungi can facilitate plant growth indirectly by reducing plant pathogens, or directly via phosphorus solubilization, nitrogen fixation, iron sequesterization by siderophores, phytohormone production (e.g. auxin, cytokinin, or giberellin), and/or enzymatic lowering of plant ethylene levels [1], [7].

Inoculation of salt-stressed plants with PGPR strains could alleviate salinity stress [8]. The uninoculated plants, compared to the inoculated plants, under soil salinity conditions had an increased antioxident activity and concentration of proline, MDA, glutathione reductase (GR) and ascorbate peroxidase.

Pseudomonas fluorescens strain TDK1 possessing ACC deaminase activity enhanced the saline resistance in groundnut plants, which in turn resulted in increased yield when compared with the groundnuts treated with Pseudomonas strains not having ACC deaminase activity.

Many PGPR strains possess the enzyme ACC deaminase [9],[6],[16] and this enzyme can cleave the plant ethylene precursor ACC, and thereby lower the level of ethylene in a developing seedling or stressed plant [17] [13]. By facilitating the formation of longer roots through the action of ACC deaminase, these growth-promoting bacteria may enhance the survival of plant seedling under various abiotic and biotic stresses [7],[20] including salinity.

Production of extracellular polysaccharides by plant growth promoting rhizobacteria as a scavenger of cations including Na+ would be one of the mechanisms employed by PGPR to

alleviate salt stress [5]. Thus, isolation and identification of PGPR strains which can produce excessive EPS under salt stress conditions would help in reducing the active concentration of Na+ in the root zone by increasing population of the excessive EPS producers [8].
Groundnut is a major oilseed crop in India with an estimated production of around 8.0 million tonnes. However, being a predominant rainfed crop, there is a number of soil-borne fungal pathogens viz. Aspergillus niger, Aspergillus flavus, Sclerotium rolfsii causing devastating havoc to groundnut in most of major groundnut growing states. Besides, increasing salinity in groundnut growing areas is also causing a potential threat to the plan of increasing plant production and productivity. Thus, development of biofertilizer packages comprising salt tolerant strains of plant growth promoting rhizobacteria and rhizobia would help in alleviating the salinity stress and thus would ensure productivity of groundnut in salinity affected areas of Gujarat.

Biological nitrogen fixation is estimated to contribute 180 x 106 metric tons/year globally [14], of which 80% comes from symbiotic associations and the rest from free-living or associative systems. These include, a) symbiotic nitrogen fixing forms, viz. Rhizobium, the obligate symbiont in leguminous plants and Frankia in non-leguminous trees, and b) N2-fixing forms such as cyanobacteria, Azospirillium, Azotobacter, Acetobacter diazotrophicus, Azoarcus, etc. Although most Rhizobium isolates can nodulate more than one host species and also several different bacterial species are often isolated from a single legume, it is only from a few legumes that the symbionts have, so far, been investigated thoroughly [23].

Non-symbiotic nitrogen fixation is known to be of great agronomic significance. The main limitation to non-symbiotic nitrogen fixation is the availability of carbon and energy source for the energy intensive nitrogen fixation process. This limitation can be compensated by moving closer to or inside the plants, viz. diazotrophs present in rhizosphere, rhizoplane or those growing endophytically. Some important non-symbiotic nitrogen-fixing bacteria include Achromobacter, Acetobacter, Alcaligenes, Arthrobacter, Azospirillum, Azotobacter, Azomonas, Bacillus, Beijerinckia, Clostridium, Corynebacterium, Derxia, Enterobacter, Herbaspirillium, Klebsiella, Pseudomonas, Rhodospirillum, Rhodopseudomonas and Xanthobacter [15]. However, all the nitrogen fixation process gets impaired under salinity stress.
Phosphate solubilizing microorganisms (PSM) not only assimilate P but a large portion of soluble phosphate is released in quantities in excess of their own requirement [4]. The most efficient PSM belong to genera Bacillus and Pseudomonas amongst bacteria and Aspergillus and Penicillium amongst fungi. PSM inoculants include species of Aspergillus, Bacillus, Escherichia, Arthrobacter and Pseudomonas which can add 30-35 kg P_2O_5 ha^{-1} [3].
Other microorganisms that are known to be beneficial to plants are the plant growth-promoting rhizobacteria (PGPR). In addition to supplying combined nitrogen by biological nitrogen fixation, certain bacteria affect the development and function of roots by improving mineral (NO_3^{-1}, PO_4^{-3} and K+) and water uptake. Considerable research is underway globally to exploit the potential of one such group of bacteria that belong to fluorescent pseudomonads (FLPs). FLPs help in maintenance of soil health, protect crop from pathogens and are metabolically and functionally most versatile [10].
Groundnut is a major crop in the Kutch district of Gujarat, large area which are and is affected by salinity. Application of beneficial microorganisms could be one of the options for alleviating salinity stress in groundnut besides other measures. The effect of PGPR on the levels of antioxidants and physiological response of groundnut under salinity stress, however, is relatively unexplored.

Thus, little is known about the effect of inoculation of salt tolerant plant growth promoting rhizobacteria on the growth, yield and nutrient uptake of groundnut under salt stress conditions vis-à-vis the possible roles played by these organisms in ameliorating the salt stress of plants. The effect of PGPR on the antioxidant status and physiological response of groundnut under soil salinity is relatively unexplored. The present investigation will address the above issues.

2. MATERIAL AND METHOD

To investigate the physiological changes that occur under different salinity levels, a trial was conducted in earthen pots by imposing three different levels of soil salinity (2, 4 and 6 EC) using cv GG2 and normal soil was used as control. Two pseudomonas culture having plant growth promoting tratits like production of IAA, HCN, ammonia, phosphate solubilisation, antifungal activity and tolerant to salinity (10% NaCl). These cultures were identified by 16s rRNA sequencing viz. *Pseudomonas aeruginosa* AMAAS57 and *Pseudomonas aeruginosa* BM6, RAPD of pseudomonas isolates were done using operon 1 to 20. Operon RAPD primer kit A containing 20 different primers (OPA1-OPA20) of 10 nucleotide length as per following details were used for developing RAPD profiles of the pseudomonas isolates(fig.7 and fig.8).

The PCR condition was 94^0 C -5 min; (94^0 C- 1min, 37^0 C- min, 72^0 C- min): 35 cycles; final extension: 72^0 C for 5 min. The PCR product was resolved in 1.2% agarose gel.
These cultures were applied to study the extent of alleviation of salinity stress, if any. Leaf samples were collected at 15-day intervals after germination and extracted in methanol was made to evaluate different parameters i.e. carbohydrate,

phenol and free amino acid related to alleviation of salinity stress.

Carbohydrate was estimated by anthrone method.

Total phenols of plants were extracted and estimated following the method described by [11].

Free amino acid was estimated using ninhydrin reagent [21].

SLA was measured by leaf area meter.

To determine leaf relative water content. About 0.1 g leaf sample was cut into smaller pids/ms and weighed to determine fresh weight. The leaf sample was floated in freshly de-ionized water for 12 hrs and weighed thereafter to determine fully turgid weight. The leaf sample was oven dried at 80^0 C for 3 days and dry weight was obtained. The relative water content (RWC) was determined using the following formula:

RWC= (fresh weight – dry weight)/(turgid weight – dry weight)X 100

The techniques used to determine membrane stability was similar to that develop by Sullivan.

3. RESULTS AND DISCUSSION:

Different plant growth promoting attributes like production of indole acetic acid, phosphate solubilisation and ammonification (Dye, 1962) were quantified.

AMAAS 57 was having important PGP traits like production of IAA (29.00µg/ml), HCN, ammonia, phosphate solubilisation(19.07 mg/25ml), ligninase, antifungal activity against S. rolsfi, A. flavus, and A. niger. And The PGP characters of BM 6 were production of IAA (15.53µg/ml), phosphate solubilisation (16.02 mg/25 ml), siderophore (0.13±.02mg/mg protein); antifungal activities against S. rolfsii, A. flavus, and A. niger.

Results showed that the level of total carbohydrate decreased gradually with increasing levels of salinity in uninoculated control (table.1) but the level of carbohydrates increased in treatment inoculated with *Pseudomonas aeruginosa* AMAAS57 with increase in salinity(Fig.5).

Table 1

Culture	ECe	Carbohydrate ug/mg	Phenol ug/mg	Free amino acid ug/mg
control	0 ds/m	6.829±0.088	46.21	5.697±0.923
	2ds/m	7.896±0.02	33.28	4.588±0.97
	4ds/m	5.298±0.047	52.94	5.51±1.28
	6ds/m	6.608±0.05	20.25	3.486±0.96
AMAAS57	0ds/m	11.29±0.063	48.02	5.521±1.35
	2ds/m	9.621±0.069	50.96	5.479±1.33
	4ds/m	8.325±0.068	47.65	4.264±1.08
	6ds/m	6.321±0.027	40.92	4.5855±0.97
BM6	0ds/m	8.28±0.080	65.12	4.759±1.25
	2ds/m	8.542±0.045	89.32	5.267±1.1
	4ds/m	5.264±0.028	60.58	3.63±1.02
	6ds/m	6.785±1.163	48.08	3.837±1.22

Table 2

Culture	ds/m	Leaf area	RWC (%)	Electrolytic leakage(%)
control	0 ds/m	5.089±0.97	68.22±0.22	20.502±0.054
	2ds/m	4.397±0.42	77.577±0.17	14.628±0.056
	4ds/m	2.943±0.031	82.837±0.031	21.8±0.041
	6ds/m	3.098±0.005	80.713±0.56	35.025±0.04
AMAAS57	0ds/m	5.614±0.51	68.957±0.06	21.868±0.19
	2ds/m	4.373±0.32	66.347±0.17	16.96±0.17
	4ds/m	3.624±0.77	74.49±0.41	28.451±0.10
	6ds/m	4.555±0.27	52.463±0.44	21.378±0.095
BM6	0ds/m	5.482±0.20	87.823±0.21	18.4±0.11
	2ds/m	5.455±0.36	81.207±0.02	27.22±0.20
	4ds/m	4.483±0.20	83.4±0.07	31.517±0.13
	6ds/m	4.209±0.48	79.573±0.03	16.796±0.16

In uninoculated treatments, the levels of phenol(Fig.4) and free amino acids (Fig.6) decreased at salinity of 2 ds/m (table.1) then increased marginally at 4 ds/m and there was a sharp decline at 6 ds/m. Application of *Pseudomonas aeruginosa* AMAAS57 increased the production of phenol and free amino acids with soil salinity of 2 ds/m (table.1) but thereafter decreased gradually with increasing salinity the decrease, however, was not as much as was observed with uninoculated control Similarly, the SLA (Fig.1) decreased with increase in salinity but application of *Pseudomonas aeruginosa* AMAAS 57 increased the SLA with increasing level of salinity. Estimation of electrolytic leakage (Fig.3) indicated that there was an increase in the leakage with increase in salinity in uninoculated treatments (table.2). However, application of *Pseudomonas aeruginosa* AMAAS57 and *Pseudomonas fluorescens* BM6 reduced the electrolyte leakage (table.2). The RWC (%) (Fig.2) increased gradually with increasing level of salinity in uninoculated controls, but application of *Pseudomonas aeruginosa* AMAAS57 lowered the level of RWC% with increase in salinity. Application of *Pseudomonas fluorescens* BM6, however, failed to lower the level of RWC% with increase in salinity.

Application of PGPR tolerant to salinity, to some extent, can alleviate the salinity stress in groundnut. These results, however, need to be validated under field conditions.

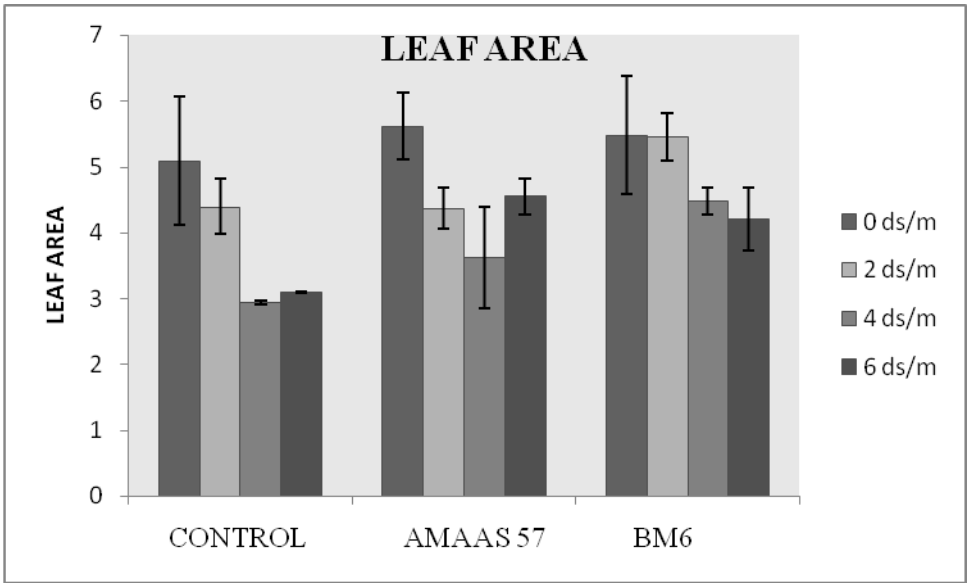


Fig.1

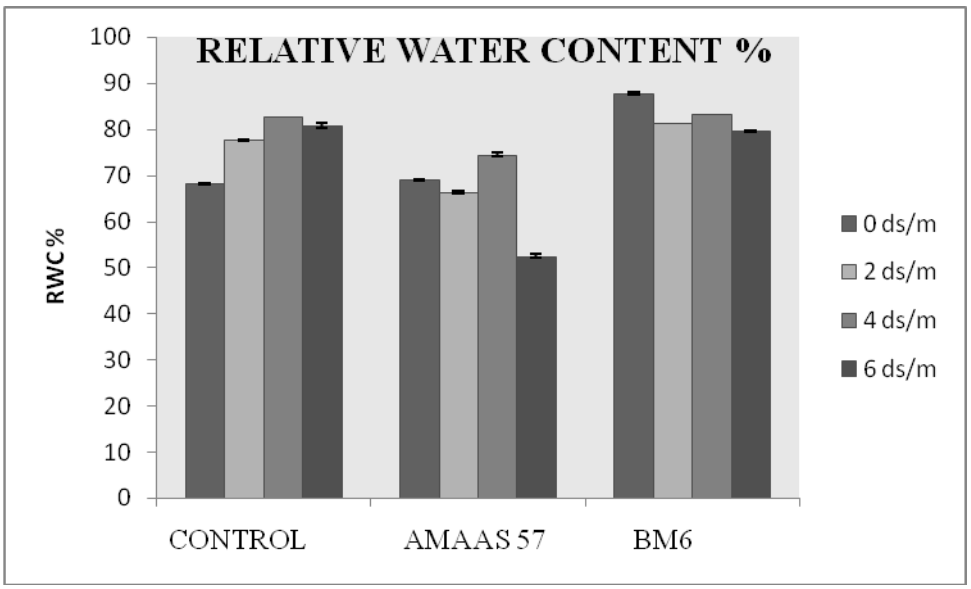


Fig.2

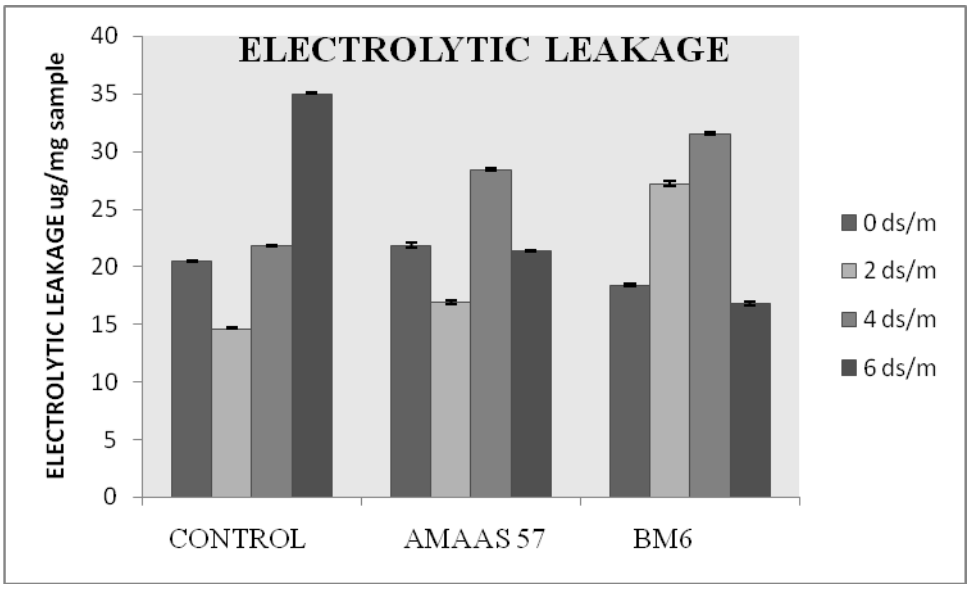


Fig.3

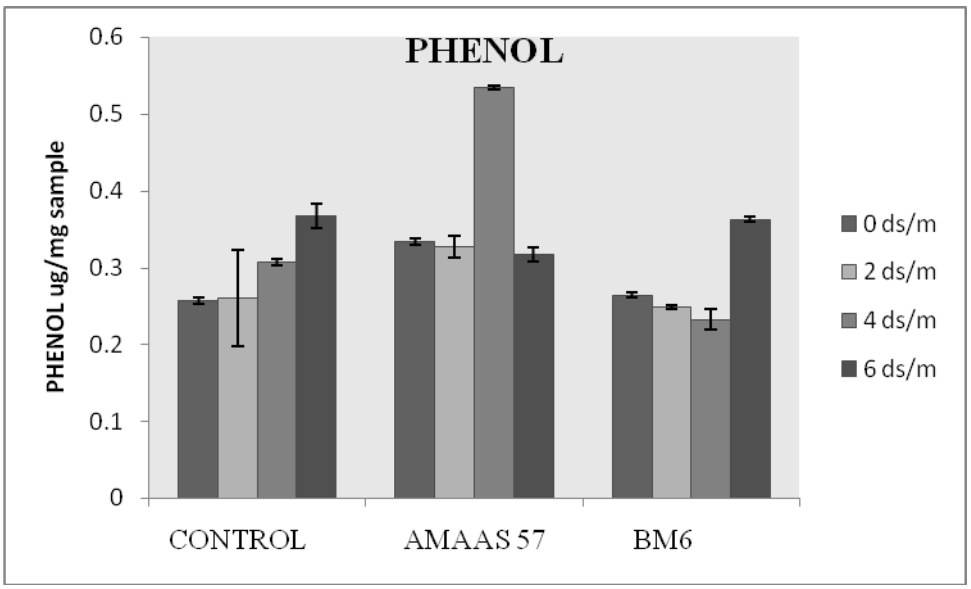


Fig.4

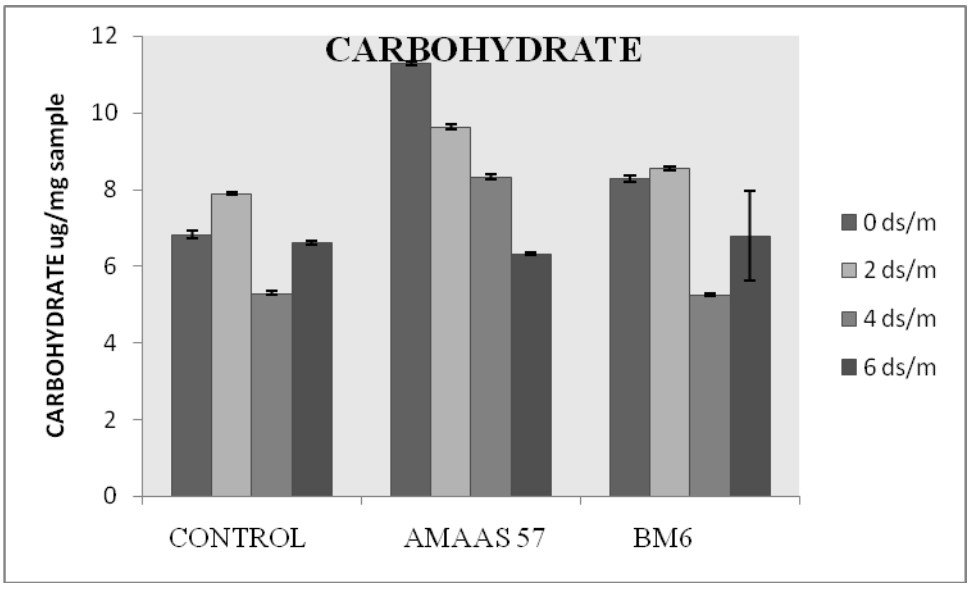


Fig.5

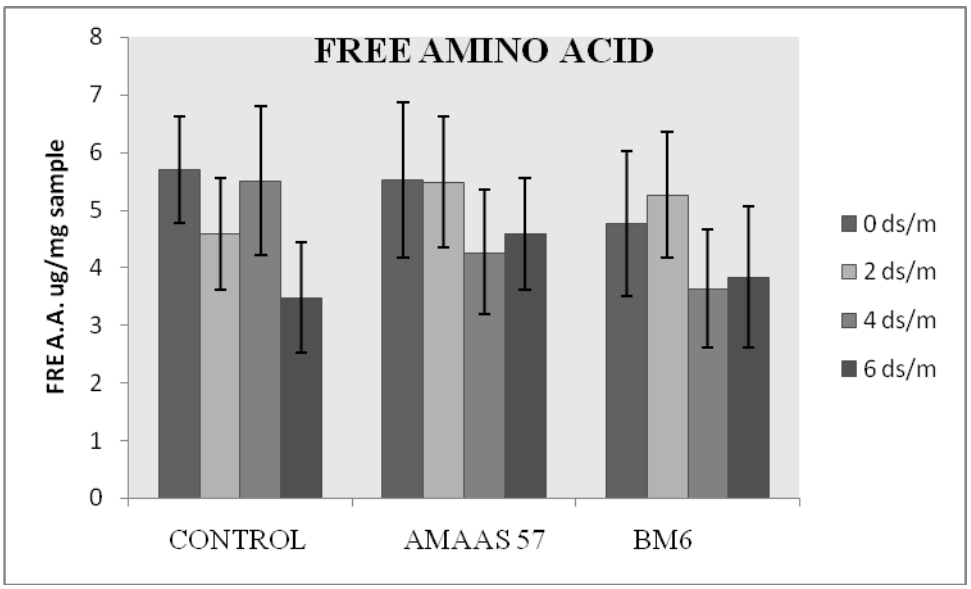
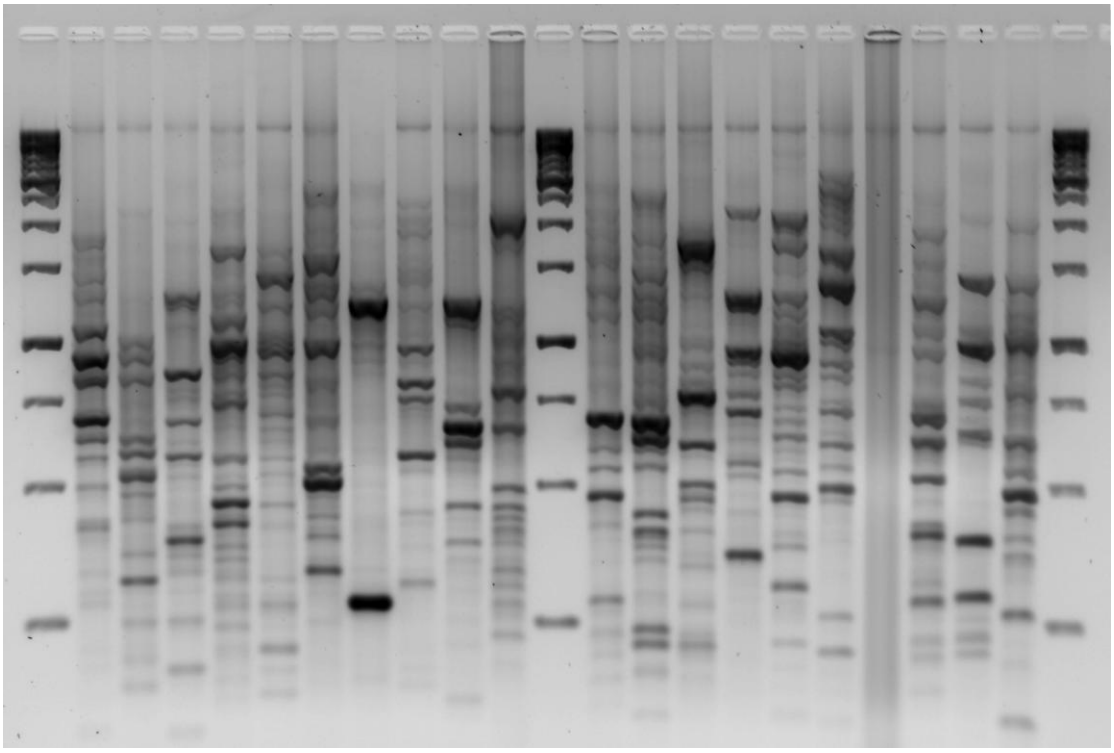


Fig.6

RAPD profile of pseudomonas AMAAS-57 using OPA-1 to OPA 20

M 1 2 3 4 5 6 7 8 9 10 M 11 12 13 14 15 16 17 18 19 20 M



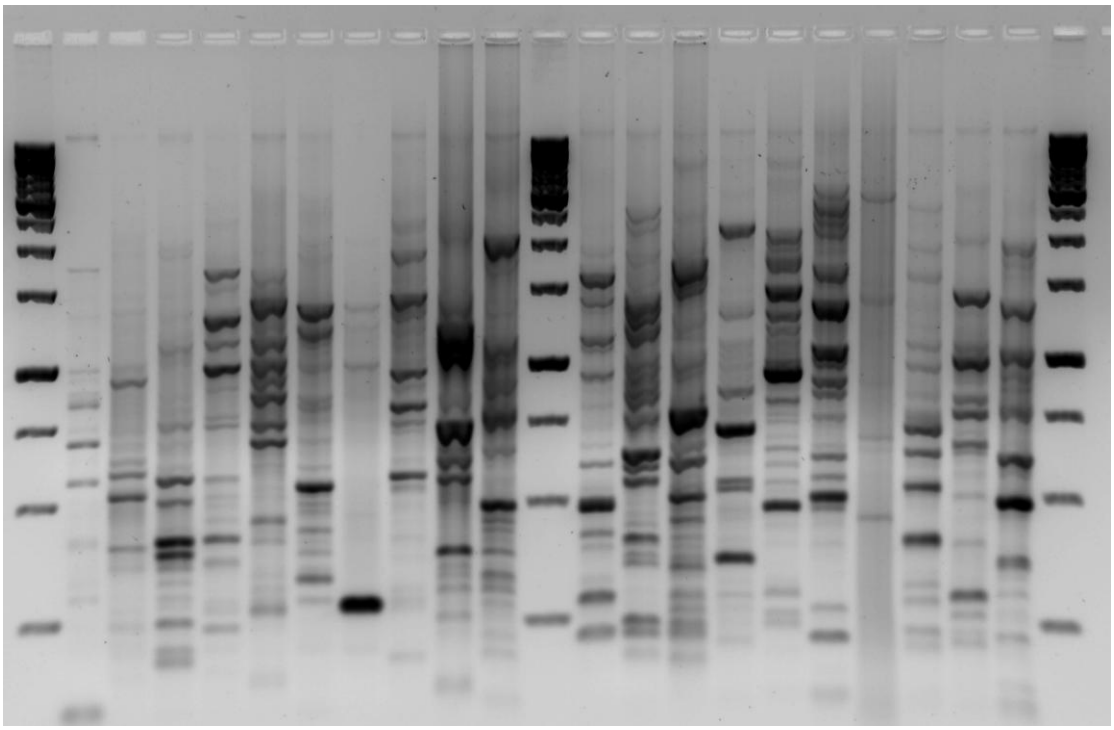
Volt –60/140-3hrs

Marker O' Gene Ruler 1kb ladder(Fermentas) (250, 500, 750, **1000**, 1500, 2000, 2500, **3000**, 3500, 4000, 5000, **6000**,8000 and 10000.)

Fig.7

RAPD profile of pseudomonas BM6 using OPA-1 to OPA 20

M 1 2 3 4 5 6 7 8 9 10 M 11 12 13 14 15 16 17 18 19 20 M



Volt –60/140-3hrs
Marker O' Gene Ruler 1kb ladder(Fermentas) (250, 500, 750, **1000**, 1500, 2000, 2500, **3000**, 3500, 4000, 5000, **6000**,8000 and 10000.)
Fig.8

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