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Microbial Diversity among *Azotobacter* strains and Efficacy Test on Chickpea *(Cicer arietinum)*

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A B S T R A C T

Non-symbiotic *(Azotobacter)* nitrogen obsession is a critical wellspring of settled nitrogen for the biosphere. Microorganisms catalyze natural nitrogen obsession with the chemical nitrogenase, which has been exceedingly moderated through development. Aside from settling nitrogen *Azotobacter* can be utilized as a plant development advancing rhizobacteria (PGPR). Soil tests were gathered from differing agrarian documented of Misrod (District Raisen) Bhopal (Madhya Pradesh). The dirt example were chosen from Rhizospheric locale of *Cicer arietinum*, speak to an extensive variety of physical and compound properties, 16S rDNA based PCR Amplification method were utilized for quality articulation. Confined protein (*Alu I*,) favored for RFLP and RAPD (OPK 20) 5'-GTGTCGCGAG-3'primer for fingerprinting and gives a high level of segregation between *Azotobacter* species. Phylogenetic examination16S-rDNA based PCR and elucidation in the wake of anticipating dendrogram changes of Azotobacter strains acquired affirms varieties among species. Presence of variable game plan on genome by reflecting slight variety coming about into foundation of decent variety among strain.

Introduction

There is awesome assorted variety in the metabolic kinds of free-living microorganisms, which are fit for BNF. This incorporates around 20 genera of non-photosynthetic oxygen consuming *(Azotobacter, Beijerinckia*) and anaerobic (*Clostridium*) microscopic organisms and around 15 genera of photosynthetic cyanobacteria (blue green growth, for example, *Anabaena* and *Nostoc*. Free-living, non-photosynthetic microbes rely upon soil natural issue as a sustenance source though the photosynthetic microorganisms may get their nourishment from the results of

photosynthesis. The nitrogen settling action of free-living, non-photosynthetic, vigorous microorganisms are firmly reliant on great dampness conditions, oxygen, and a natural sustenance source. Anaerobic delegates *(Clostridium)* prevail in field and waterlogged soils and soil totals where dampness conditions and natural substrates are accessible yet oxygen supply to the microenvironment of the microscopic organisms is extremely confined. The underlying foundations of such plants may supply the nitrogen-settling microorganisms with a moderately high and managed supply of nourishment (photosynthetic), or, in other words restricted supply in the rhizosphere of generally plants.

Free-living nitrogen-settling anaerobic microbes are available and settle nitrogen in the intestinal substance of an assortment of creatures (herbivores) and furthermore man. Nitrogenase activity is generally quite low and its significance in terms of satisfying nutritional requirements of the host appears doubtful. Most nitrogen-fixing bacteria (typically members of the genus *Rhizobium*) form symbiotic associations with leguminous plants, where they are provided with nutrients by the plant and simultaneously protected from oxygen, which poisons the enzyme required for nitrogen fixation (nitrogenase *Azotobacter* species are Gram-negative, aerobic soil-dwelling bacteria.

They are typically polymorphic, i.e. of different sizes and shapes. Their size of the cells ranges from 2-10 µm long and 1-2 µm wide Old cells tend to form thick-walled, optically retractile cysts, which have capsules consisting largely of alginates and other polysaccharides, enhancing their resistance to heat, desiccation and adverse environmental conditions. (Malviya *et al.*, 2017) Improvements in sub-atomic science based procedures have prompted quick and dependable instruments to portray microbial network structures and to screen their elements under in situ conditions RAPD remains for Random Amplification Polymorphism DNA. RAPD responses are PCR responses, however they open up portions of DNA, which are basically obscure to the researcher (irregular).

PCR is utilized to open up a known grouping of DNA. Along these lines, PCR prompts the intensification of a specific section of DNA Bacterial provinces in Petri dishes yet at last obtained an arms stockpile of sub-atomic DNA based methods that enable them to watch the living beings in their indigenous habitats. Microbial creature are considerably more differing then we at any point envisioned (Cheryl kuske *et al.*, 2005) see how tremendous that assorted variety may be microscopic organisms are basic for breaking down and reusing supplements at a worldwide scale.

The method for distinguishing these new microbes includes removing and sequencing one quality from every one of the microorganisms in an ecological example. The 16S qualities are the marker. Novel devices to describe populaces in soil' created and enhanced devices to examine nitrogen-settling microorganisms in soil, with an accentuation on the free-living (Asymbiotic) populace. Chickpea (*Cicer arietinum L.*) (Malviya *et al.*, 2018) is an important leguminous crop of Indian subcontinent. It has been grown in India for times immemorial. The rhizobia comprise a diverse group of organisms that bring forth hypertrophic growth on the roots of legume plants to form a new organ, the root nodule, which they reside in to fix nitrogen. Although the root nodule structure can develop without bacterial infection but infection and organogenesis normally proceed simultaneously. Nodule development is under

control of specific nodulation (nod) signal(s) produced by rhizobia in response to the secretion of phenolic compounds by host plant. A marker quality of nitrogen-settling microorganisms *(nifH*) was utilized as a sub-atomic marker to consider the decent variety, and by means of the discovery of mRNA, additionally the action of these life forms in soil. This methodology permits to connect auxiliary data about a microbial network specifically to the visibly recognizable action of nitrogen obsession.

This methodology gives a vital apparatus to connect the structure-action hole that speaks to a noteworthy test to current microbial environment. Various strategies were created to propel this innovation: To evaluate the assorted variety of this utilitarian quality and its appearance, nucleic acids (DNA and RNA) must be removed from soil a basic advance on which every single downstream investigation depend. Enhanced conventions for powerful extraction of DNA and RNA from soil were created.

Various investigations duplicates of this quality creating 16s quality. Trouble in concentrate such a significant number of life forms they have created network fingerprinting methods, basically coding at the issue at a much lower goals (Malviya *et al.,* 2012). This sub-atomic unique finger impression examination can be utilized to screen bacterial many-sided quality the relative plenitude and elements of these infinitesimal networks at a scene level in reality.

Another outskirt of science is developing that joins science the investigation of normal items from already uncultured soil microorganisms. The methodology Involves straightforwardly getting to the genomes of soil living beings that can't be, or have not been, refined by secluding their DNA, cloning it into cultural creatures and screening the resultant clones for the generation of new synthetic substances. The fervor encompassing this new field lies in the huge decent variety of obscure soil miniaturized scale verdure and the substance lavishness that they are thought to contain. In order to develop bioinoculants, both metabolic fingerprinting and genetic fingerprinting have been used to study the diversity among *Azotobacter* spp. Isolated different district of Madhya Pradesh.

Materials and Methods

Sampling site

Soil samples were collected from 10 different agricultural filed of Misrod (District Raisen) Bhopal (Madhya Pradesh). Rhizospheric region of *Cicer arietinum*, the soil sample were selected to represent a wide range of physical and chemical properties. (Malviya *et al.*, 2011)

Isolation of free-living nitrogen fixing bacteria

Dilution series was made ranging from$10^{-1}$to 10^{-9} Colonies growing on Jensen's agar medium. The procedure followed for isolation of *Azotobacter* strains from soil, purification of culture and authentication test were as described Vincent (1970). The colony were isolated is off-white, white, yellow and some are in lemon color, consistency is soft, some are opaque, and shiny with regular and irregular marginal.

Germination of seed

The investigation was spread out in a Complete Randomized Design with four replication. The parameters considered were germination percent (%), germination list, shoot length (cm), root length (cm), seedling

length (cm), seedling crisp weight (g), seedling dry weight (g), force file I and II. Perceptions were recorded on five haphazardly chosen seed for every one of the parameters from every replication.

The execution of natural and inorganic development controllers was assessed on premise of research facility germination test by gathering distinctive germination parameters viz., germination per cent(%), germination file, shoot length (cm), root length (cm), seedling length (cm), new weight of seedling (gm), dry weight of seedling (gm), seed life record I, and seed power file II. These parameters were measurably examined and basic contrasts were determined.

DNA isolation

For total genomic DNA isolation, culture of *Azotobacter* was grown for 24d at 28^0 C in Jensen's agar medium. Cells were pelleted several times by centrifugation at 10000rpm/10min. Add 1ml of 50mM Tris (pH-8.0) and 1ml of 50mM EDTA and dissolved the pellet.

Freeze it in deep freezer at –20°C for 30min and add 0.5 ml of 250mM tris base (H-8.0) 10µl of 10 mg/ml. Lysozyme and then place on ice for 45 min.

Add 0.5ml of 0.5% SDS, 0.5ml of 50mM Tris (pH-8.0), 0.5ml of 0.4 m EDTA, add 10 µl of 1mg/ml of proteinase K place in water bath at 50^0C for 60 min. Put 3ml of phenol & centrifuge at 10000rpm /15 min. Transfer the top layer to new tube (Avoid interference). Add 0.1volumes of 3M sodium acetate mix gently and then add 2 volume of 95% ethanol mix by inverting spool out DNA & Transfer to 1.5 ml Eppendorff. Put 0.5 ml 50mM tris.5) 0.5ml 1mM EDTA. Dissolve overnight by rocki8ng at 4°C. Add 10µl RNAse and incubated for 1 hour at 37°C Extract with equal volume of chloroform mix by inverting take top layer in new tube after centrifuged at 10000 rpm/5min. Add 0.1volume 3M sodium acetate (mix gently) and the add 2 vol. 95% ethanol. Spool out DNA & Dissolve in 60µl TE buffer (pH -8.0) the concent5ration and the purity of DNA were estimated spectrophotometrically at 260 and 280nm (Malviya *et al.,* 2018)

PCR of the isolated DNA

Two different Oligonucleotide primers, obtained from (Pharmacia Biotech ltd.) were chosen arbitrarily. All the primers used in this work were 10 nucleotides in length and has a good GC content of 70 %. Primers sequences (5'to 3') were as follow FDI (5' CCG AAT TCG TCG ACA ACA GAG-3') and RDI (5'-CCC GGG ATC CAA GCT TAA G -3') DNA amplification was done in thermal cycler (Mini Cycler, TM MJ Research PTC-100).

With the following temperature profile an initial Denaturation (94°C for 5 minutes), 30 cycles of Denaturation (30seconds a 94°c), annealing (50°C for 40 seconds) and extension (72°C for 90 seconds) and a final extension (72°C for 7 minutes). For every PCR reaction, a negative control (no template DNA) and a positive control (Template, DNA giving amplified product) were in variably maintained. The amplified product was run on a 0.8% agarose gel along with 1Kb MW marker, at a constant voltage and visualized under UV light (Uvitch Gel Documentation system) (Malviya *et al.,* 2018).

RFLP (Restriction fragment length polymorphism)

Amplified product (17ul of the PCR mix) was digested with 1unit of restriction endonucleases *(Alu I)* according to the manufacturer's recommendations).

Restriction Fragments were separated on a 2.5% agarose gel and stained with ethidum bromide. A 100 bp DNA Ladder was used as molecular weight marker.

RAPD analysis random amplified polymorphic DNA

The procedure described by (Williams et, al., 1990) with minor modification was used for caring out PCR reaction to produce RAPD profiles. Amplification of DNA fragments was carried out by the Polymerase Chain reaction were carried out using the same DNA as used for 16s rDNA. The reaction mixture consisted of 200µM of dNTP mix 15 pico mole of primer, 1U/µl of Tag DNA polymerase and 25mM $MgCl_2$. (OPK 20) \5'-GTGTCGCGAG-3' was selected because it was able to differentiate among isolates.

Scoring and data analysis

The extent of every limitation part or RAPD piece was computed and the profiles of the strains were analyzed groups of sections shorter than 90 bp were not mulled over since they were not settled reproducibly. Each band created with a specific limitation chemical or RAPD groundwork was scored over every one of the examples.

Information was entered utilizing 0-1 grids in which every watched band or characters were recorded. The RFLP example and RAPD profile of each confine was assessed doling out character state "1" was given for a band, which could be unmistakably and reproducibly recognized in the RFLP or RAPD examination. The date lattice subsequently created was utilized to figure Jacard's similitude coefficient for each match insightful correlation. The coefficient was figured after recipe (Jaccard, 1908). The unweighted combine aggregate built Dendogram from the closeness network. Technique with number juggling means averaging (UPMGA) (Sneath and Sokal, 1973). With the end goal to test the integrity of attack of group investigation, cophenetic esteem network were ascertained and contrasted and the first closeness lattice that were UPMGA bunched by utilizing the NTSYS-pc examination bundle (Version 1.3, Exeter programming Setauket, N.Y.) (Malviya *et al.*, 2012)

Results and Discussion

Hereditary decent variety of *Azotobacter* species from agribusiness field of Raisen area of M.P. is completed in the wake of accommodating properties of soil from various testing destinations. *Azotobacter* is a variety, or, in other words set up free-living nitrogen settle additionally (Asymbiosis) with rhizospheric locale which is controlled by quality item. The dirt qualities impact the event of rhizospheric district and natural nitrogen obsession. Test got from locales A1 A2 A3 altogether broke down in the research center with existing strategy.

Isolation of *Azotobacter*

Tests acquired from rhizospheric region of *Cicer Arietinum,* and exchanged to pre-disinfected holders and named by locales. The way of life was developed and keeps up on Jensen's medium at 28^0C for 20 mins. Culture qualities of *Azotobacter* are exhibited in Table 1. Besides morphological investigations by gram recoloring are analyzed and gram negative bars are seen. Development of all separated strain was performed in presterlized stock and strains were assigned as moderate developing species (Table 1).

All the gathered *Azotobacter* strains were indistinguishable however displayed variety in development and physiological qualities. All grayish white, yellow bacterial states and

demonstrated existences of *Azotobacter* species none of the confine gave positive outcome for gelatin liquefaction test. Social and biochemical test fill in as sign to recognize the dirt microorganism. Following the premise of biochemical corroborative records the secluded *Azotobacter* strains from farming field were subjected to hereditary decent variety investigation.

Hereditary substance of life form uncovers its situation in natural decent variety general the microscopic organisms including free-living nitrogen fixers. Rhizospheric soil additionally occupies bacterial decent variety straightforwardly affected by topographical and natural elements. Portrayals of people are administered by articulation of hereditary pool which thus underpins position of people in transformative progressive system. Alteration of hereditary setup requires the advancement in species involving useful living space.

Hereditary decent variety of *Azotobacter* species from horticulture field of Raisen area of M.P. is done subsequent to accommodating properties of soil from various testing destinations. *Azotobacter* is a variety, or, in other words builds up free-living nitrogen settle likewise (Asymbiosis) with rhizospheric locale which is dictated by quality item. The dirt qualities impact the event of rhizospheric district and natural nitrogen obsession. Test got from destinations A1 A2 A3 altogether broke down in the lab with existing strategy.

Characterization of *Azotobacter*

Tests got from rhizospheric area *Cicer Arietinum*, and exchanged to pre-cleaned holders and named by destinations. The way of life was developed and keeps up on Jensen's medium at 28^0C Culture attributes of *Azotobacter* are introduced in Table 1. Moreover morphological investigations by gram recoloring are analyzed and gram negative bars are seen. Development of all separated strain was performed in presterlized soup and strains were assigned as moderate developing species (Table 1).

All the gathered *Azotobacter* strains were indistinguishable however shown variety in development and physiological attributes. The grayish, white, yellow bacterial states and demonstrated existences of *Azotobacter* species. None of the detach gave positive outcome for gelatin liquefaction test. Social and biochemical test fill in as sign to recognize the dirt microorganism. Biochemical corroborative records the detached *Azotobacter* strains from horticultural field were subjected to hereditary decent variety investigation.

Hereditary substance of creature uncovers its situation in natural assorted variety general the microbes including free-living nitrogen fixers. Rhizospheric soil likewise possesses bacterial decent variety straightforwardly impacted by geological and organic variables. Portrayals of people are represented by articulation of hereditary pool which thusly bolsters position of people in transformative chain of command. Adjustment of hereditary setup requires the advancement in species involving useful territory.

Biochemical confirmatory records the isolated *Azotobacter* strains from agriculture field were subjected to genetic diversity analysis Genetic content of organism reveals its position in biological diversity general the bacteria including free-living nitrogen fixing nitrogen fixing rhizospheric soil also occupied bacterial Morphological characterization indicated a close resemblance of these isolates to *Azotobacter spp.* BIOLOG cataloguing divided them into two main groups, but amplified ribosomal DNA restriction analysis clustered the isolates from the four regions

having different soil types into different district of Madhya Pradesh.

Germination record

The information uncovered that speed of germination demonstrates critical distinction among the different medications. The speed of germination ran from 11.58 to 15.00.The most extreme speed of germination (15.00) was seen in JM-8 (GA3 20 ppm) which was observed to be essentially predominant pursued by JM-7 (GA3 10 ppm) 14.75 though, least speed of germination was recorded in charge to (11.58). The most extreme speed of germination with GA3 may be because of its impact in early germination and expanded percent germination. The outcomes are in similarity with discoveries of Rajamanickam and Anbu (2001) (Fig. 1 and 2).

The root length, shoot length and seedling length changed altogether in every one of the medications as contrasted and control during the investigation. It was biggest in joined impacts of bio manures over control and the individual impact of every alteration independently. Most noteworthy root length, precise advancement of plants and seeds shoot length and seedling length with use of *Azotobacter*, PSB and FYM in individual and in consolidated impacts of these demonstrates that these small scale atoms helps in the possibility of plant to endure well even in worry of dampness, effectiveness of supplements take-up and even mechanical help to the plant against rapid breezes and tempests in stormy season; likewise shoot length during testing shows the proficiency of speed of germination and furthermore better development and leaves improvement.

Despite the fact that maize has all around created vascular packs and stomata for effective physiological procedures including admission of climatic CO2 for usage of photosynthetic pathways of C4 cycles. These three characters are generally significant for generation purpose of perspectives as they are fundamentally and emphatically connected with development and yield parameters and aides in foundation of sound plants in fields oppressed for creation.

Germination rate was exceptionally impacted because of different medicines over control. It may be ascribed because of increasingly efficient improvement of sound fetuses in seeds and the amassing of supplements in seeds which helps in improving the level of germination and which were qualified according to standard of germination endorsed by affirmation principles. Different medicines appeared beneath 90% germination rate.

Maize is cross pollinated crop and planted in more extensive dispersing consequently poor germination will influences the plant stand per unit region coming about poor collect. Higher the estimations of germination in T4 for example with PSB pursued by *Azotobacter* (93.67%) and PSB+ FYM blends (92.67%) individually. These qualities satisfy the germination guidelines recommended for maize. Different medicines demonstrated this worth less consequently 90% and non-critical numerically over control. Higher the germination % for PSB, *Azotobacter* and consolidated impact of PSB+FYM showed that PSB, *Azotobacter* and blend of PSB+FYM is useful in solid embryonic advancement and furthermore in dynamic translocation of nourishments integrated during photosynthesis in leaves to kernels (Table 2).

The seed treated with GA3 20 ppm was observed to be the most appropriate development controller for germination and energy of chickpea since it uncovered

predominant execution in a large portion of the parameter viz., germination percent (97.50%), germination file (15.00), shoot length (17.87cm), root length (15.89cm), seedling length (33.07 cm), seedling new weight (7.71 g), seedling dry weight (1.54g), life file I (3223.03) and force file II (149.93).

DNA isolation and PCR

Genomic DNA of 24 isolated culture of *Azotobacter* were ornamental of 0.8 % agarose gel electrophoresis and purity of DNA were estimated spectrophotometrically at 260and 280nm (Fig. 3). A novel molecular strategy for studying microbiological diversity is based on restriction digestion of 16S rDNA sequence were separated on a 2.5% agarose gel and stained with ethidum bromide and visualized under UV light. The 16S rDNA of 24 isolated strains war PCR amplified with universal primers (5'-CCG AAT TCG TCG ACA ACA GAG-3') and (5'-CCC GGG ATC CAA GCT TAA G-3').

Table.1 Biochemical characterization of free-living nitrogen fixing bacteria

S.No	Strain	Jensen media	Gram staining	Bacteria colonies	Hoffer's alkaline medium –ve	Glucose peptone –ve	Ketolactose –ve	Liquefi-ction of gelatin	Incubation period at for period in 28±1in Hrs.	S/M Slow/ moderate
1.	JM1	+	-ve rod	Off white	–ve	–ve	–ve	–ve	96	**S**
2.	JM2	+	-ve rod	white	–ve	–ve	–ve	–ve	96	**S**
3.	JM3	+	-ve rod	Off white	–ve	–ve	–ve	–ve	96	**S**
4.	JM4	+	-ve rod	Off white	–ve	–ve	–ve	–ve	96	**S**
5.	JM5	+	-ve rod	Off white	–ve	–ve	–ve	–ve	96	**S**
6.	JM6	+	-ve rod	Yellow	–ve	–ve	–ve	–ve	96	**S**
7.	JM7	+	-ve rod	Off white	–ve	–ve	–ve	–ve	96	**S**
8.	JM8	+	-ve rod	Off white	–ve	–ve	–ve	–ve	96	**S**
9.	JM9	+	-ve rod	Yellow	–ve	–ve	–ve	–ve	96	**S**
10	JM10	+	-ve rod	White	–ve	–ve	–ve	–ve	96	**S**
11	JM11	+	-ve rod	White	–ve	–ve	–ve	–ve	96	**S**
12	JM12	+	-ve rod	White	–ve	–ve	–ve	–ve	96	**S**
13	JM13	+	-ve rod	Lemon	–ve	–ve	–ve	–ve	96	**S**
14	JM14	+	-ve rod	Off white	–ve	–ve	–ve	–ve	96	**S**
15	JM15	+	-ve rod	Off white	–ve	–ve	–ve	–ve	96	**S**
16	JM16	+	-ve rod	Off white	–ve	–ve	–ve	–ve	96	**S**
17	JM17	+	-ve rod	Lemon	–ve	–ve	–ve	–ve	96	**S**
18	JM18	+	-ve rod	Cream	–ve	–ve	–ve	–ve	96	**S**
19	JM19	+	-ve rod	Cream	–ve	–ve	–ve	–ve	96	**S**
20	JM20	+	-ve rod	Cream	–ve	–ve	–ve	–ve	96	**S**
21	JM21	+	-ve rod	Off white	–ve	–ve	–ve	–ve	96	**S**
22	JM22	+	-ve rod	Off white	–ve	–ve	–ve	–ve	96	**S**
23	JM23	+	-ve rod	Off white	–ve	–ve	–ve	–ve	96	**S**
24	**JM24**	**+**	**-ve rod**	**Off white**	**–ve**	**–ve**	**–ve**	**–ve**	**96**	**S**

Table.2 Effect of treatments on germination%, root length, shoot length, seedling length, dry weight and seed vigour index of winter chickpea

Strain	Treatment	Vigour index	Germination%	Root length cm	Shoot length cm	Seedling length cm	Dry weight
JM	Control	1261.06	83.67	11.67	7.00	17.67	15.02
JM-1	*Azotobacter*	1408.79	93.67	11.66	8.97	21.00	14.04
JM-2	FYM	1201.01	83.00	11.67	10.00	21.67	17.67
JM-3	Ph+Azo+FYM	1493.52	84.00	17.00	15.33	32.33	17.78
JM-4	PSB	1566.32	96.33	14.00	14.33	28.33	16.26
JM-5	PSB.+ Azo	1264.51	86.67	16.00	9.67	25.33	14.59
JM-6	PSB + FYM	1423.41	92.67	15.67	13.00	28.67	15.36
	S.Ed. ±	-	1.53	1.38	0.90	1.53	0.17
	C.D. at 5%	-	3.33	2.99	1.96	3.32	0.37

RFLP (Restriction fragment length polymorphism)

The PCR products were individually restricted with endonucleases *(Alu-I) showed* the discriminative banding pattern ranging from 100- 9000 bp (Fig. 4) Similarity and dissimilarity co-efficient were analyzed by UPGMA (Malviya *et al.*, 2012) Cluster analysis among the isolates JM18, JM17, JM7, JM8, JM22, JM10, JM9, JM14, JM6, JM24, JM23, JM12, JM11, JM4, JM3, JM1, JM2 and JM20 showed 100% similarity and JM18-JM7 showed 20%, dissimilarity however strain JM6-JM22 showed 24% dissimilarity among the strain JM24-JM3 showed 25% the ornamentation of strains between JM1-JM12 were shows 26% dissimilarity among the isolates (Fig. 5)

RAPD analysis random amplified polymorphic DNA

(OPK 20) 5'-GTGTCGCGAG-3' was selected because it was able to differentiate among isolates (Malviya *et al.*, 2012) (Fig. 6).

Fig.1 Germination of *Cicer arietinum* in the presence of *Azotobacter* culture

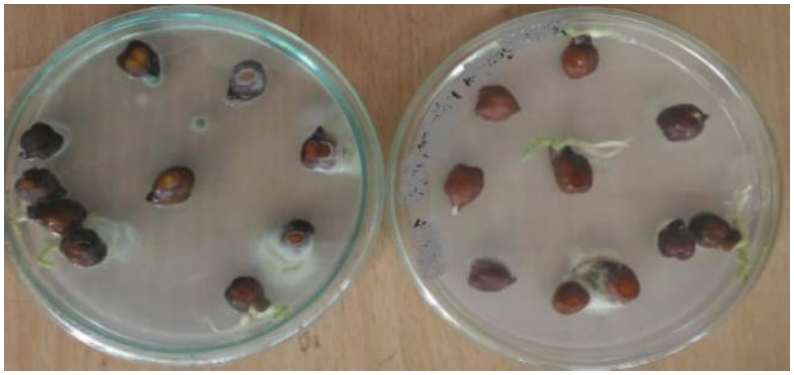

Fig.2 Germination of rhizome of *Cicer arietinum* in the presence of *Azotobacter* culture

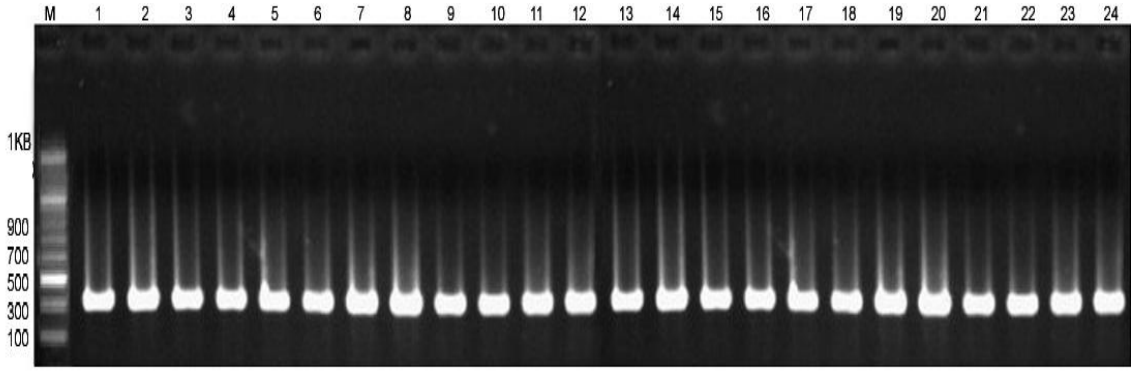

Fig.3 Isolated DNA of *Azotobacter* species on 2% Agarose gel

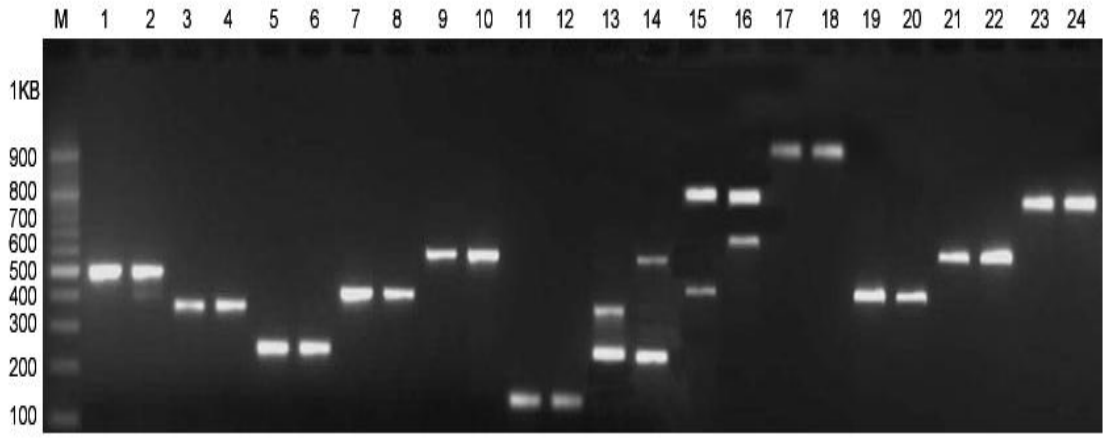

Fig.4 RFLP amplification of *Azotobacter* species on 2% Agarose gel

Scoring and data analysis

The unweighted combine aggregate built Dendogram from the closeness organize. Method with number shuffling mean averaging (UPMGA) (Sneath and sokal 1973). With the ultimate objective to test the honesty of assault of gathering examination, cophenetic regard arrange were found out and differentiated and the main closeness grid that were UPMGA clustered by using the NTSYS-pc assessment pack (Version 1.3, Exeter programming Setauket, N.Y.) (Malviya *et al.*, 2012).

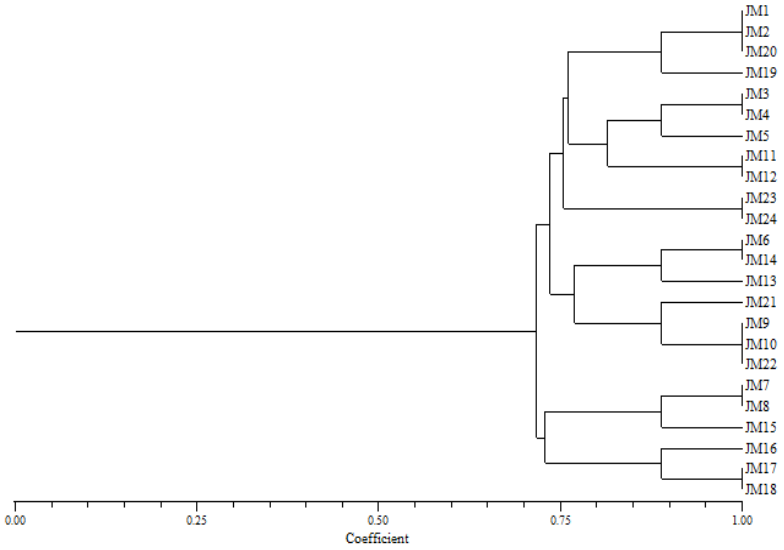

Fig.5 Cluster analysis of RFLP pattern with NTSYs software

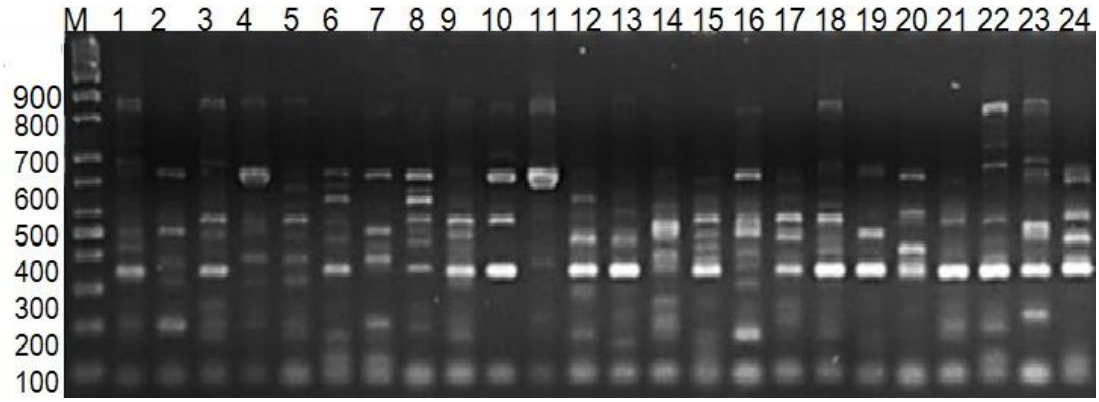

Fig.6 RAPD amplification of *Azotobacter* species on 2% Agarose gel

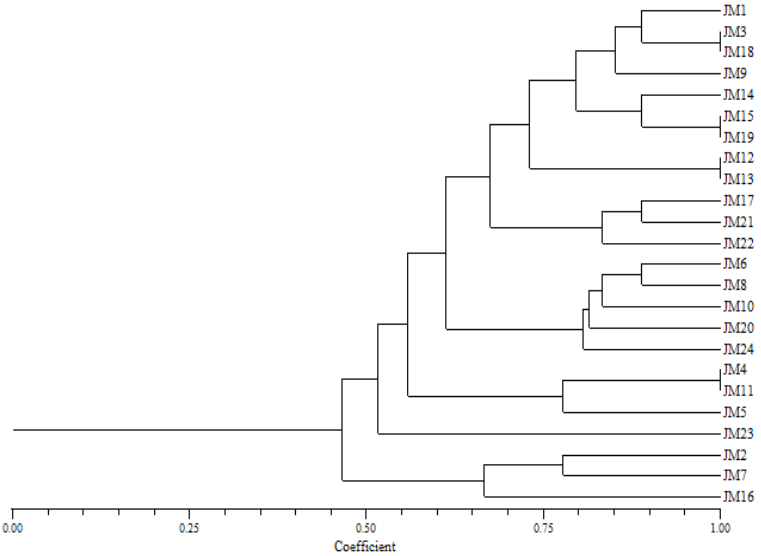

Fig.7 Cluster analysis of RAPD pattern with NTSYs software

Likeness and difference co-effective were broke down by UPGMA Cluster investigation among the detaches 2,7 and 16 are half comparative among all disconnects 4 and 11, 12 and 13, 03 and 16, 15 and 19 are gave 100% similitude appeared to one another. The likeness coefficient demonstrates the conveyance of comparability record with in the separates indicated a potential (Fig. 7).

Experimentation recommends that PCR can effectively estimate the development natural surroundings and geographic beginning. Investigations of (Iruela *et al.,* 2002) in the variety *Cicer* and developed *chickpea* utilizing mix of RAPD and ISSR markers of 26 promotions including kabuli and desi types were utilized. (Rao *et al.,* 2007) created RAPD and ISSR fingerprinting in developed chickpea and its wild ancestor *Cicer reticulatum* L.

They finished up ISSR examination as dependable traits for estimation of hereditary decent variety than of RAPD. (Fatemeh *et al.,* 2013) utilized ISSR markers to unique mark hereditary decent variety and preservation of landrace chickpea from north-west of Iran. ISSR markers are adequately scored for hereditary assorted variety investigation of different harvest plants. (Amirul *et al.,* 2015) evaluated hereditary decent variety among gathered purslane promotions. In view of their evaluation they suggested not many of the promotion that can be utilized as guardians in future reproducing program.

In the present experiment, the Chickpea being a self-pollinated crop has a tight hereditary base. Taking a gander at gigantic chickpea germplasm accessible and to diminish expenses of field experimentation, genotype screening utilizing PCR based markers is an essential for reproducing program. In view of hereditary root and decent variety list viz. prescribed to be chosen as a parent in future rearing projects for chickpea. More endeavors are expected to screen chickpea promotions which can evaluate hereditary decent variety inside the *Cicer* species to recognize first class hereditary stocks.

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