Plant Science Research 45 (1&2) : 92-98, 2023

Plant Science Research

ISSN 0972-8546

Analysis of genetic diversity among fluorescent Pseudomonads isolated from groundnut rhizosphere

Padma Gurucharan Jogi[1], Bosetty Anjana[1], E. C. Surendranath Reddy[2] and P. Osman Basha[1Ψ]

[1] Department of Genetics and Genomics, Yogi Vemana University, Kadapa, Andhra Pradesh, India.
[2] Department of Biotechnology, Yogi Vemana University, Kadapa, Andhra Pradesh, India.

ARTICLE INFO

Article history:
Received : 2O October 2023
Revised : 10 November 2023
Accepted : 30 November 2023

Keywords:

Fluorescent *Pseudomonads*
Genetic diversity
Groundnut
PGPR
RAPD

ABSTRACT

Fluorescent Pseudomonads as plant Growth Promoting Rhizobacteria (PGPR) has played a significant role in plant development with decrease in the severity of several fungal diseases. The present research work focus on characterization of genetic diversity of isolated 24 fluorescent Pseudomonads isolated from different rhizosphere of the groundnut in the Rayalaseema region of Andhra Pradesh. Genetic diversity was analyzed by RAPD markers and among the studied 12 primers; eight primers produced 100% polymorphic bands while the OPC12 primer showed a monomorphic band. A total number of 87 amplified bands were identified, of which 80 polymorphic bands were noted and the PIC value ranged from 0.12 to 0.36 for polymorphic markers.The dendrogram analysis divided the isolates into two major and two minor clusters. The knowledge gathered here about the genetic diversity of fluorescent *Pseudomonads* associated with the ground soil rhizosphere helps to understand their role and potential use in sustainable agriculture.

1. Introduction

The genus *Pseudomonas* comprises a group of omnipresent microbes found in the various ecological habitats, such as soil, water, sediments, fungi, animals, plants and humans surfaces possibly just because of their easy nutritional necessities (Brown *et al*., 2012; Rainey *et al*., 1993; Vela *et al*., 2006; Scales *et al*., 2015; Jogi *et al*., 2020). Fluorescent *Pseudomonas* spp. can be visually distinguished from other *Pseudomonas* by their capability to synthesize a yellow-green fluorescent compound (Jogi *et al*., 2020). Fluorescent Pseudomonads strain can be isolated from plant surroundings and are usually stated as plant growth-promoting rhizobacteria (PGPR) (Jogi *et al*., 2020). These Fluorescent Pseudomonads PGPR species have a biotechnological interest because of their capability to influence plant hormonal balance (Kang *et al*., 2006) and improve plant vigor by decreasing the effects of plant-pathogens.

The Random amplified polymorphic DNA (RAPD) is widely used for the SCAR molecular markers development, diversity assessment, and identifying markers linked with traits of interest in microbes and plants. Primers are designed randomly about 10 bp and the technique is used in organisms where the DNA sequence is unknown (Williams *et al*., 1990). Irrespective of the source or age of the organism, the RAPD patterns are fairly accurate and exceptionally helpful for germplasm characterization, estimation of diversity, and genetic resource conservation programs (Welsh and McClelland, 1990). The RAPD markers have been utilized successfully to estimate the genetic variability of fluorescent Pseudomonads (Patel *et al*., 2018; Megha *et al*., 2007).

The genetic diversity assessment of several plant species was reported very frequently by several researchers. The RAPD molecular marker technology facilitates the genetic characterization of *Ocimum* (Patel *et al*., 2015), *Changiums myrnioides* (Fu *et al*., 2003), coffee species (Mishra *et al*., 2014), and simultaneous identification of *T.*

Ψ *Corresponding author; Email:* osmanbasha@yahoo.co.in

cordifolia, E. officinalis and *T. terestris* (Shinde, 2007). Molecular and genetic analysis of *Urginea indica, Rauvolfia serpentine,* and *Rauvolfia tetra phyla* L., using RAPD markers has been studied (Padmalatha and Prasad, 2006 & 2007; Ruan *et al*., 2008). The RAPD technique has been generally used for genetic assessment of wild plants (Khasa and Dancik, 1996; Manica-Cattani *et al*., 2009).

The RAPD molecular markers are simple and resourceful and have been employed toassess the genetic variation at the molecular level in fluorescent Pseudomonads. In general, establishing the fluorescent Pseudomonads genetic diversity from the groundnut crop rhizospheres through genetic markers is a critical step in determining the frequency of distribution of these isolates in various locations of the Rayalaseema region. To keep these in view the genetic variation among the 24 isolates of fluorescent *Pseudomonads* using selected 12 primers RAPD were characterized.

2. Materials and methods

2.1. Bacterial strains

The fluorescent Pseudomonads were isolated from soil samples of groundnut rhizosphere at the Rayalaseema region of Andhra Pradesh, India (Jogi *et al*., 2020). These isolates were maintained at glycerol (50%) stocks at -80 0C.

2.2. Genomic DNA isolation

Genomic DNA was isolated from fluorescent Pseudomonads following the protocol described by Ausubel *et al.* (1992) with few modifications.

2.3. RAPD-PCR

Twelve random primers, namely OPA-01, OPA-11, OPA-17, OPD-01, OPD-02, OPD-03, OPE-02, OPE-04, OPF-10, OPF-11, OPF-14, and OPC12 (Operon technologies Inc.), were evaluated to construct the dendrogram. PCR mix was freshly prepared by mixing 10 X assay buffer (2.5µl), 2.0mM $MgCl_2$ (2.0µl), 10mM dNTPs (0.5µl), 0.5 units of Taq DNA polymerase (0.2µl), 0.6 ìm primer (2µl) and sterile milli Q water (15.8µl) in 0.2 ml PCR tubes. To this 23µl of PCR mixture, 2µl of genomic DNA (30µg) was added. Amplification was carried out by following the PCR program; 5min of initial denaturation at 94°C, followed by 40 cycles of denaturation at 94°C for 1min, annealing at 37°C for 2min, extension at 72°C for 2min and final extension step at 72°C for 10min. The amplified PCR products were separated on 1.5% agarose gel electrophoresis with 1X TBE buffer and the amplified products were visualized and the RAPD-banding pattern was documented using Gel documentation instrument.

2.4. Data Analysis

The RAPD-PCR and gel electrophoresis experiments were repeated at least three times to verify the banding pattern and certain reproducible bands on the gels were evaluated for data analysis. A score of "1" was given for the presence and "0" for the absence of bands. All the reproducible bands of 12 RAPD gels were scored for the corresponding band as presence (1) and absence (0) in all 24 Fluorescent pseudomonads samples. The binary data consisting of "1" and "0" generated for all alleles or bandswereevaluated for the Jaccard's similarity coefficient values and genetic similarity using unweighted pair group arithmetic mean (UPGMA) program using NTSYSpc 2.02j software. The Polymorphism Information Content (PIC) value was calculated following the formula; PIC = 2fi(1-fi), Where, fi is the frequency of the amplified allele (band present) and (1-fi) is the frequency of the null allele (band absent) (Roldán-Ruiz *et al*., 2000; Soengas *et al*., 2006).

3. Results

A total of 24 fluorescent Pseudomonads were isolated from ground cultivating areas of Andhara Pradesh (Jogi *et al*., 2020). All of the isolates were screened against *S. rolfsii* (data not shown) and these effective isolates have been used forgenetic diversity studies.

3.1. RAPD-PCR analysis

Genomic DNA of 24 PGPR bacterial isolates was successfully amplified with 12 RAPD oligonucleotide primers. The amplified products were analyzed to detect polymorphism and genetic diversity among the fluorescent Pseudomonads. The number of amplified products obtained was specific to each primer, and it ranged from 1 to 12 (Table 1). A total of 87 alleles were scored, of which 80 (91.95%) were polymorphic (Table 1). Out of 12 oligonucleotide primers, 8 primers viz. OPA-01, OPA-11, OPD-01, OPD-02, OPE-04, OPF-10, OPF-11, and OPF-14 produced 100% polymorphic bands,while OPA-17, OPD-03, and OPE-02 primers gave both mono and polymorphic band patterns and one primer (OPC-12) produce monomorphic banding pattern (Table 1).The amplification patterns revealed a high level of polymorphism and multiple DNA amplification products with 5 to 12 alleles were noted, except OPC-12 (Table 1). The maximum number of alleles was observed in OPD-1 and OPF-10 (Table 1). The OPA-01 and OPA-17primers produced 9 alleles, OPA-11 primer produced 8 alleles, whereas OPD-03, OPE-02, OPE0-4, OPF-11, and OPF-14 primers produced only 5 alleles (Table 1).The polymorphism information content (PIC) was calculated for each primer and it's ranged from 0.5 to 0.36, whereas OPC-

12 gives monomorphic bands and had a PIC value of zero (Table 1). The highest PIC values were observed in OPD-01 and OPF11 followed by OPA-01 and OPA11 (Table 1).

The dendrogram revealed two major clusters and two minor clusters and the major clusters were subdivided into different sub-clusters (Figure 1). The Jaccard's similarity coefficient matrix was examined for highest and lowest similarity coefficient values for each fluorescent Pseudomonad strains (Table 2). Among them, PGY1, PGY5, PGY3, PGY4, PGY6, PGY13, PGY17, and PGY18 strains belonged to the major cluster-I (Figure 1). The PGY5 strains showed the highest similarity coefficient with PGY1, PGY3, PGY4, and PGY6 stains (Table 2), hence all these strains were associated in sub-cluster-Iof the cluster-I (Figure 1). The PGY13 and PGY18 showed the highest similarity coefficient with PGY17, and the PGY17 showed the highest similarity coefficient with PGY18, hence these three were included in thesub-cluster-IIof cluster-I (Table 2 and Figure 1). The PGY5 had the second-highest similarity coefficient with PGY13, PGY17, and PGY18 strains; therefore all these eight strains were included in cluster-I. The PGY5 strain forms a link between the sub-clusters-I and sub-cluster-II; hence these two sub-clustersfall in the major cluster-I (Table 2 and Figure 1).

Another major cluster-II consisting of PGY7, PGY9, PGY8, PGY11, PGY14, PGY16, PGY15, PGY12, PGY19, PGY24, and PGY20 strains, and these were classified into three sub-clusters (Figure 1). The PGY7, PGY9, PGY8, and PGY11 formsub-cluster-Iof the cluster-II (Figure 1). The analysis of similarity coefficient values suggested that the PGY7 had the highest similarity coefficient with PGY9 and the PGY8 had the highest similarity coefficient with PGY11 (Table 2). The PGY9 had the second-highest similarity coefficient with PGY11 (Table 1). Due to these similarity coefficient values, these four strains fall in sub-cluster-I (Figure 1). The PGY14 had the highest similarity coefficient with PGY16, while PGY 15 and PGY16 showed the highest similarity coefficient with PGY14; hence these three strains were classified as sub-cluster-II of the major cluster-II (Figure 1). The strain PGY24 had the highest similarity coefficient value with PGY19 and the PGY12 and PGY19 had the second-highest similarity coefficient values with PGY24 (Figure 1). Consequently, these three PGY12, PGY19, and PGY24 form the sub-cluster-III of the major cluster-II (Figure 1).

The other isolates PGY21 & PGY22 and PGY10 & PGY23 are classified into two small clusters (Figure 1). The PGY21 and PGY22 strains showed the highest similarity coefficient values with each other and belong to a separate minor cluster-III of the dendrogram (Figure 2). The PGY23 strain showed the lowest similarity coefficient value with PGY2, PGY4, PGY5, PGY11, PGY12, PGY17, and PGY24; hence the PGY23 strain grouping away from all other clusters (Table 1 and Figure 1). The PGY1 to PGY6 samples were collected from the Anantapur district were placed in sub-cluster-I of cluster-I and have a similarity coefficient value of more than 0.75 (Figure 1). Three of the PGY13, PGY17, and PGY18 isolates collected from the Kadapa district were categorized in the sub-cluster-II of the cluster-I (Figure 1). Out of 12 isolates collected from the Anantapur and Kadapa districts of the Rayalaseema region, nine isolates were grouped into thecluster-I of the dendrogram. Whereas cluster-II was formed with three sub-clusters mainly samples collected from three districts (Figure 1). The isolates collected from Kurnool (PGY7, PGYPGY9, PGY8, and PGY11) and Kadapa (PGY14, PGY16, and PGY15) were categorized into two sub-clusters of the dendrogram (Figure 1). The two isolates (PGY19 and PGY24) isolated from the Chittoor district were placed in sub-cluster-III of thecluster-II (Figure 1).

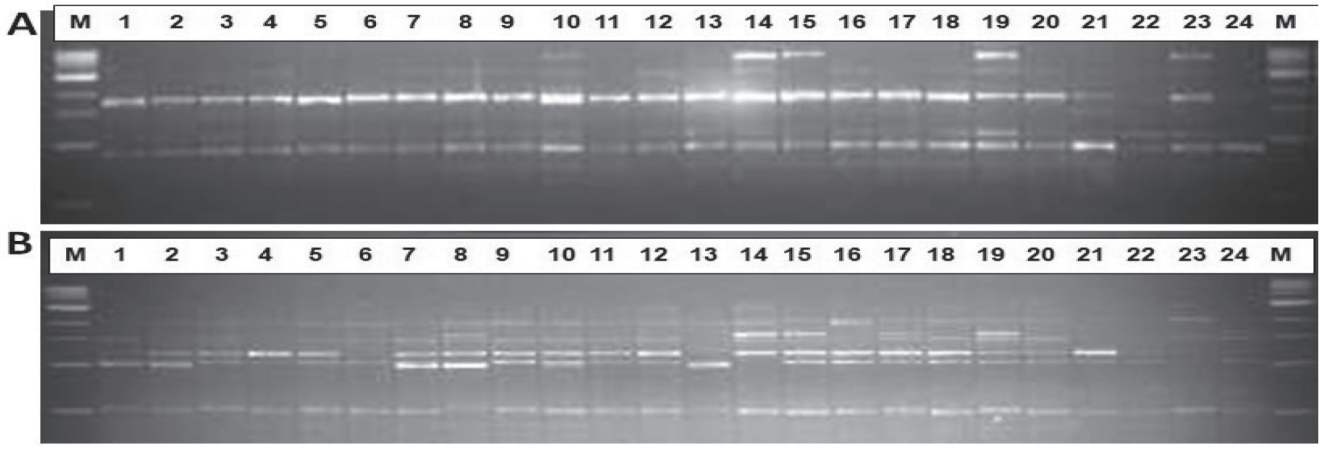

Figure 1: Random Amplified Polymorphic DNA (RAPD) amplification profile of 24 isolated fluorescent Pseudomonadsstrains with primer OPA-3 (A) and OPA-10 (B); M: 1 kb DNA marker.

Table1

List of primer and degree of polymorphism of RAPD-PCR amplification among the 24 isolate fluorescent Pseudomonads; PB-Number of polymorphic bands; MB-Number of monomorphic bands; PIC- Polymorphism Information Content.

S.No	Primer code	The primer nucleotide sequence (5' to 3')	Total alleles	PB	MB	% PB	% MB	PIC
1	OPA-01	CAGGCCCTTC	9	9	0	100	0	0.34
2	OPA-11	CAATCGCCGT	8	8	0	100	0	0.31
3	OPA-17	GACCGCTTGT	9	7	2	77.78	22.22	0.12
4	OPD-01	ACCGCGAAGG	12	12	0	100	0	0.36
5	OPD-02	GGACCCAACC	11	11	0	100	0	0.27
6	OPD-03	GTCGCCGTCA	5	3	2	60	40	0.20
7	OPE-02	GGTGCGGGAA	5	3	2	60	40	0.05
8	OPE-04	GTGACATGCC	5	5	0	100	0	0.28
9	OPF-10	GGAAGCTTGG	12	12	0	100	0	0.25
10	OPF-11	TTGGTACCCC	5	5	0	100	0	0.36
11	OPF-14	TGCTGCAGGT	5	5	0	100	0	0.24
12	OPC-12	TGTCATCCCC	1	0	1	0	100	0

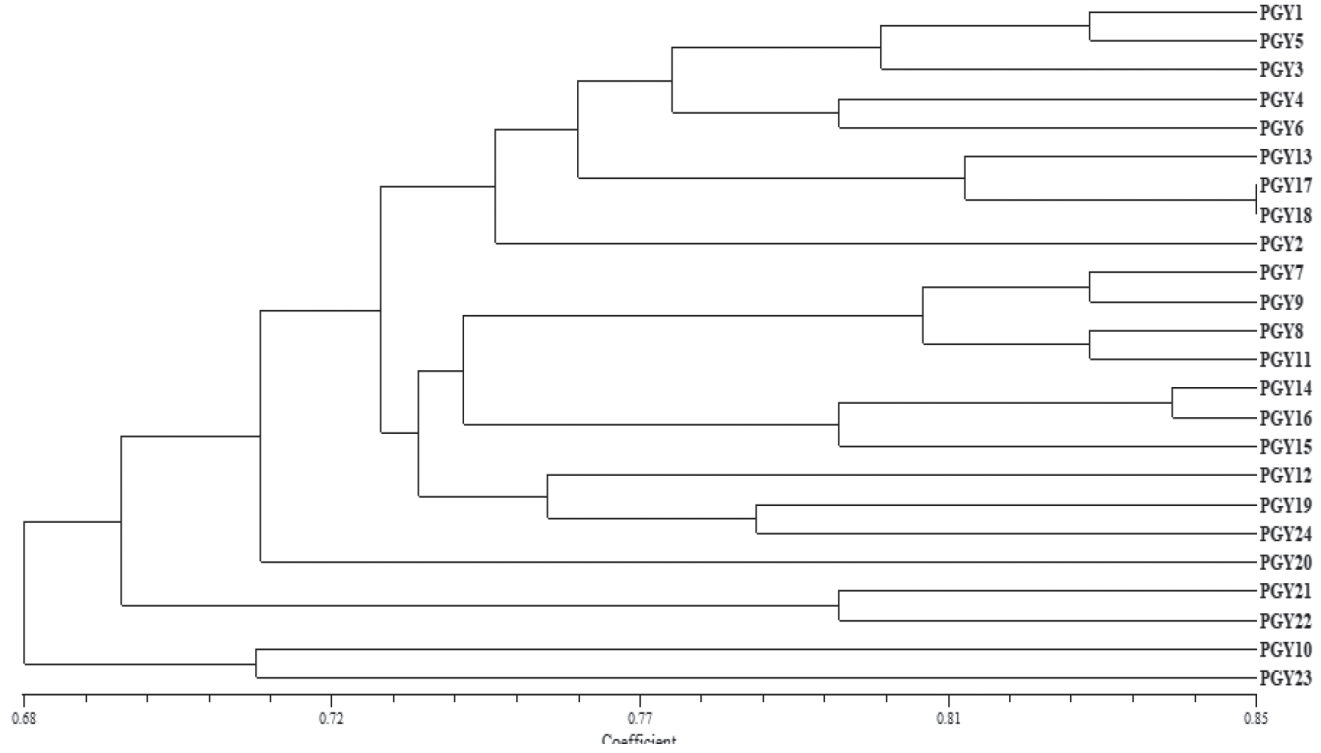

Figure 2: Dendrogram derived from unweighted pair group arithmetic mean (UPGMA) program using NTSYS pc 2.02j software showing the relationship among 24 fluorescent Pseudomonads bacterial isolates.

4. Discussion

In the present study, RAPD technique was successfully utilized for rapid characterization of 24 fluorescent *Pseudomonas* isolates of the Rayalaseema region of the State of Andhra Pradesh, India. The 24 isolates of fluorescent Pseudomonads genetic variability were categorized into different clusters and sub-clusters. Among the different molecular markers, Random Amplified Polymorphic DNA (RAPD) technique issuitableand is easy to execute as it does not have the need for any DNA sequence information of any species (Weder, 2002). Due to its technical simplicity, the use of RAPD as molecular markers for taxonomic and systematic analyses of plants and bacteria has increased. In-spite of the difficulty of reproducibility of RAPD molecular markers until in recent times, they are the well-known markers in terms of price and are widely used to study all living species genetic diversity. The RAPD molecular markers sequence information homology is restricted to the 10 bases of each amplification product. RAPD has been used for the estimation of genetic diversity in various endangered plant species and bacteria (Wang *et al*., 2005; Lu *et al*., 2006; Rayar *et al*., 2015).

A total of 12 different operon series primers were used to assess the 24 isolates and a total of 87 alleles were scored, of which 80 (91.95%) were polymorphic (Table 1). Out of 12 oligonucleotide RAPD primers, 8 primers produced 100% polymorphic bands, while three primers gave both mono and polymorphic alleles. These banding patterns revealed diverse raging of amplification patterns and a significant level of polymorphism. The total number of alleles noted for all markers were ranged from one to twelve and the maximum was noted by OPD-1 and OPF-10 primers (Table 1).Rameshkumar *et al*. (2011) RAPD data of Pseudomonad isolates isolated from rhizosphere of Sugarcane and its banding pattern revealed amplification product size ranged from 100 to 2,500 bp with three pgs RAPD primers and the dendrogram analysis differentiate the majority of isolates and they were grouped in different clusters. Asadhi *et al*. (2013) studied the genetic variability of Fluorescent Pseudomonads using RAPD markers and identified 8 to 17 numbers of amplified bands and the product sizes ranging from 100 to 3000 bp. The studied eight RAPD primers produced a total number of 99 amplified products with 100% polymorphism (Asadhi *et al*., 2013). The similarity coefficient of the sugarcane germplasm was ranged from 0.43 to 0.91 when thirty-five RAPD markers were used for genetic diversity assessment (Patel *et al*., 2018). Rayar *et al*. (2015) analyzed 17 isolates and reported 63.85% polymorphic bands with a genetic similarity ranged from 0.11 to 0.73. Singh (2015) studied and determined the genetic variability of plant growth-promoting rhizobacterial (PGPR) fluorescent Pseudomonads associated with chickpea (*Cicer arietinum* L.) rhizosphere using RAPD markers and concluded the existence of a low level of genetic variability in the species at 50% similarity level.

The dendrogram output categorized the fluorescent Pseudomonads isolated from different locations of the Rayalaseema region into two major clusters and two minor clusters. The results demonstrated thepresence of a degree of genetic variation among them. The genetic distance similarity matrix values for the 24 isolates ranged from 0.575 to 0.851. The highest similarity coefficient valuesof 0.851 were found between PGY17 and PGY18 and the lowest value of 0.575 was reported between PGY10 and PGY20. It is reported that the genetic diversity analysis of *Pseudomonas fluorescence* isolates collected from rice (*Oryza sativa*) rhizosphere from Southern India had an amplification range from 100 bp to 2 Kb with a similarity of approximately 25% to 95%. The diversity assessment of PGPR fluorescent Pseudomonads strains isolated from turmeric rhizosphere from Tamil Nadu, India, concluded that the RAPD amplified products varied from 100 to 2500 bp with a total of 92 alleles and a total of seven primers produced 100% polymorphic bands out of the 15 RAPD primers (Prabhukarthikeyan *et al*., 2019). Out of the 94 total alleles observed, 82 alleles were polymorphic and higher PIC was calculated for OPA01 followed by OPF10 (Prabhukarthikeyan *et al*., 2019). The dendrogram was constructed using a total of six primers which produced 296 polymorphic bands and the eightisolates were classified into two major classes and these *P. fluorescen*ce isolates were collected from the root zone of citrus (Koche *et al*., 2020).

Extensive investigation on fluorescent Pseudomonads isolates genetic diversity is essential to evaluate the intra-species diversity analysis. These diversity studies at the intra-species level will be useful in the conservation and development strategies. Based on the molecular genetic studies and similarity index, all isolates collected from Anantapur district were classified in sub-cluster-I with a similarity coefficient value of more than 0.75, and three isolates collected from Kadapa district were categorized in the sub-cluster-II of major cluster-I. The cluster-II mainly consists of three sub-clusters and most of the samples were collected from the Kurnool, YSR Kadapa and Chittoor districts of the Rayalaseema region. The samples collected from Anantapur and Chittoor districts were placed distantly from each other. The isolates collected from Anantapur and few isolates of Kadapa may be analogousto each other and the few isolates of Kurnool and Kadapa may be analogous to each other. It is also apparent that three of the isolates

collected from the Chittoor district were separated from most of the isolates. Genetic diversity assessment of strains from the different forest areas of the Western Ghats of Uttara Kannada District, Karnataka, revealed 37 strains as Pseudomonas fluorescence, 13 isolates as *Pseudomonas aeruginosa*, and two isolates as *Pseudomonas aureofaciens* (Megha *et al*., 2007).

References

Asadhi, S., Reddy, B.V., Sivaprasad, Y., Prathyusha, M., Murali Krishna, M.T., Kumar, K.V.K. and Reddy, K.R. (2013). Characterisation, genetic diversity and antagonistic potential of 2,4-diacetylphloroglucinol producing Pseudomonas fluorescens isolates in groundnut-based cropping systems of Andhra Pradesh, India.Archives of Phytopathology and Plant Protection.6:1-12.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (1992). Current protocols in molecular biology. New York: Greene Publishing Association; Wiley-Interscience V.1.

Bartish, I.V., Garkava, L.P., Rumpunen, K. and Nybom, H. (2000). Phylogenetic relationship and differentiation among and within population of *Chaenomeles* Lindl. (Rosaceae) estimated with RAPDs and isozyme. Theor. Appl. Genet. 101: 554-563.

Brown, S.D.and Utturkar, S.M.*et al*. (2012). Twenty-one genome sequences from Pseudomonas species and 19 genome sequences from diverse bacteria isolated from the rhizosphere and endosphere of Populusdeltoides. J. Bacteriol. 194: 5991–5993.

Fu, C., Qiu, Y. and Kong, H. (2003). RAPD analysis for genetic diversity in *Changium smyrnioides* (Apiaceae), an endangered plant. Bot. Bull. Acad. Sin. 44: 13-18.

Jogi, P.G., Jyothi, L.N., Kumar, K.V.K., Basha, P.O. and Reddy, E.C.S.(2020).Morphological and biochemical characterization of fluorescent *Pseudomonads* from groundnut rhizosphere.Current Trends in Biotechnology and Pharmacy. 14 (3): 340-346.

Kang, B.R., Yang, K.Y.*et al*. (2006). Production of indole-3-acetic acid in the plant-beneficial strain *Pseudomonas chlororaphis* O6 is negatively regulated by the global sensor kinase GacS. Curr.Microbiol. 52: 473-476.

Khasa, P.D. and Dancik, B.P.(1996). Rapid identification of white-Engelmann spruce species by RAPD markers. Theoretical and Applied Genetics. 92(1):46-52.

Koche, M., Gade, R.M., Kothikar, R.B. and Tekade, A. (2020). Biochemical studies on genotypic characterization of *Pseudomonas fluorescens* isolates by PCR-RAPD analysis. IJCS. 8(2): 2915-2917.

Manica-Cattani, M.F., Zacaria, J., Pauletti, G., Atti-Serafini, L. and Echeverrigaray, S. (2009). Genetic variation among South Brazilian accessions of *Lippia alba* Mill. (Verbenaceae) detected by ISSR and RAPD markers. Brazilian Journal of Biology. 69(2):375-380.

Megha, Y., Alagawadi, A. and Krishnaraj, P. (2007). Diversity of fluorescent pseudomonads isolated from the forest soils of the Western Ghats of Uttara Kannada. Current Science. 93(10): 1433-1437.

Micheli, M.R., Bova, R., Pascale, E. and D'Ambrosio, E.(1994). Reproducible DNA fingerprinting with the random amplified polymorphic DNA (RAPD) method. Nucleic Acids Res. 22: 1921-1922.

Mishra, M.K., Nishani, S., Gowda, M., Padmajyothi, D., Suresh, N., Sreenath, H. and Raghuramulu, Y. (2014). Genetic Diversity Among Ethiopian Coffee (*Coffea arabica* L.) Collections Available In Indian Gene Bank Using Sequence Related Amplified Polymorphism Markers. Plant Breeding and Seed Science.70(1):29-40.

Padmalatha, K. and Prasad, M.N.(2006).Genetic Diversity in *Rauvolfia tetraphylla* L. using RAPD Markers.Journal of Plant Biotechnology. 33(2):139-145.

Padmalatha, K. and Prasad, M.N.(2007). Inter and intrapopulation genetic diversity of *Rauvolfia serpentina* (L.) Benth. exKurz, an endangered medicinal plant, by RAPD analysis. Medicinal and Aromatic Plant Science and Biotechnology.1(1):118-123.

Patel, P., Rajkumar, B.K., Parmar,P., Shah, R. and Krishnamurthy, R. (2018). Assessment of genetic diversity in *Colletotrichum falcatum* went accessions based on RAPD and ISSR markers. Journal of Genetic Engineering and Biotechnology. 16(1):153-159.

Patel., H.K, Fougat, R.S., *et al*. (2015). Detection of genetic variation in *Ocimum* species using RAPD and ISSR markers.3 Biotech. 5: 697-707.

Prabhukarthikeyan, S.R., Keerthana, U., Kumar Y.M. and Raguchander, T.(2019).Comparative analysis of genetic diversity among; fluorescent pseudomonads using RAPD and ISSR fingerprinting.Res. J. Biotech.14(7): 86-93.

Raaijmakers, J.M. and Weller, D.M.(2001). Exploiting genotypic diversity of 2,4-diacetylphloroglucinol-producing Pseudomonas spp.: characterization of superior root-colonizing *P. fluorescens* strain Q8r1-96. Appl. Environ. Microbiol. 67: 2545-2554.

Rainey, P.B., Brodey, C.L. and Johnstone, K. (1993). Identification of a gene cluster encoding three high-molecular-weight proteins, which is required for synthesis of tolaasin by the mushroom pathogen *Pseudomonas tolaasii*. Mol. Microbiol. 8: 643-652.

Rameshkumar, N., Ayyadurai, N., Kayalvizhi1, N. and Gunasekaran, P. (2011). Genotypic and Phenotypic Diversity of PGPR Fluorescent Pseudomonads Isolated from the Rhizosphere of Sugarcane (*Saccharum officinarum* L.). J. Microbiol. Biotechnol. 22(1): 13-24.

Rayar, J.K., Arif, M. and Singh, U.S. (2015). Relative efficiency of RAPD and ISSR markers in assessment of DNA polymorphism and genetic diversity among Pseudomonas strains. Africal journal of Biotechnology. 14(13): 1097-1106.

Ruan, Y., Mo, R.H., Li, M., Huang, L.Q., Luo, Y, Li, X. Y., Zhou, J. and Wu, Y. S. (2008). Molecular characters of *Centella asiatica* found with RAPD technology. Zhongyaocai Journal of Chinese medicinal materials. 31(7): 970-973.

Scales, B.S., Erb-Downward, J.R., LiPuma, J.J. and Huffnagle, G.B. (2015). Draft genome sequences of five *Pseudomonas fluorescens* subclade, I., and II Strains, isolated from human respiratory samples. Genome Announc. 3:e00837-15.

Shinde, V.M., Dhalwal, K., Mahadik, K.R., Joshi, K.S. and Patwardhan, B.K. (2007). RAPD Analysis for Determination of Components in Herbal Medicine.Evid Based Complement Alternat Med.4 (Suppl 1):21-23.

Singh, B.P. (2015). Molecular and functional diversity of PGPR *fluorescent Pseudomonads* based on 16S rDNA-RFLP and RAPD markers. Journal of Environmental Biology.36(5):1169-1178.

Soengas, P.,Velasco, P., Padilla. G., Orda, A. and Cartea, M.E. (2006). Genetic Relationships Among *Brassica napus* Crops Based on SSR Markers.Hort Science. 41(5): 1195-1199.

Tidke, S.A., Kiran, S. and Harke, S.N. (2014).Analysis of genetic diversity in 20 cotton germplasm lines using Random Amplified Polymorphic DNA maker.Asian Journal of Plant Sciences. 12(4-8):184-189.

Vela, A.I., Gutiérrez, M.C., *et al*. (2006). *Pseudomonas simiae* sp. nov., isolated from clinical specimens from monkeys (*Callithrix geoffroyi*). Int. J. Syst. Evol. Microbiol. 56 (Pt 11): 2671-2676.

Wang, Z.S., An, S.Q., *et al*. (2005). Genetic structure of the endangered plant *Neolitseasericea* (Lauraceae) from the Zhoushan archipelago using RAPD markers.Ann. Bot. 95: 305-313.

Weder, J.K.P. (2002). Influence of experimental conditions on the reproducibility of RAPD-PCR identification of legumes and cereals. Food Sci. Technol.-LEB. 35: 233-238.

Welsh, J. and McClelland, M. (1990). Fingerprinting genomes using PCR with arbitrary primers.Nucleic Acids Research. 18(24): 7213-7218.

Williams, J.G.K, Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531-6535.