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RAPD Analysis of Powdery Mildew Mycoparasite *Ampelomyces quisqualis* and its Genetic Polymorphism

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ABSTRACT: Powdery mildew diseases caused by Erysiphales fungi are major threat in fruits and vegetable cultivation. The different genera of powdery mildew fungi are serving the host for mycoparasitic fungi and which are diversified in nature. To study the polymorphism of mycoparasite *viz., Ampelomyces quisqualis,* the randomly amplified polymorphic DNA RAPD-PCR analysis was carried out to assess the genetic diversity of twenty isolates of *A. quisqualis* from various powdery mildew hosts in different geographical regions of Tamil Nadu. A total of ten oligonucleotide random primers were used (OPA 1 to 5, OPB01, and OPF 2 to 4) in this study. The operon series primers (OPA 1, 3, 5), OPB 3 and 10 effectively differentiated and showed reproducible banding patterns. The Unweighted Pair Group Method of Arithmetic Means (UPGMA) dendrogram constructed genetic distance produced 2 main clusters (3 isolates in cluster I and 17 isolates in Cluster II). Cluster I comprises of AQTNAU-DST01, 02 and 03. The isolates of AQTNAU-DST8 to16, AQTNAU-DST18 and 20 are separated into sub cluster 1; AQTNAU-DST04 to 07, 18 and 20 in sub cluster 2 clubbed together and formed Clade II in Hierarchy cluster analysis. The Hierarchical Cluster Analysis (HCA) based clustering of isolates showed 58-86% similarity with reference to RAPD. Non-metric Multi-dimensional scaling (MDS) plot resulted that AQTNAU-DST01, 02 and 03 are clustered in same line. Finally, our results indicate that RAPD are sensitive and give reproducible results for assaying the genetic variability among the *Ampelomyces* spp. and RAPD marker was reliable and could be utilized to establish the genetic relatedness of *Ampelomyces* spp.

Keywords: Variability studies, RAPD analysis, *Ampelomyces* isolates.

INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench), generally known as lady's finger or bhendi, is a popular vegetable all over the world. It is a member of the Malvaceae family. It is a major vegetable crop in India, and its notable varieties are commercially farmed in many areas of the world for its delicious taste and high nutritional content (Benchasri, 2012). In India, a variety of fungal, bacterial, and viral diseases have been recorded causing damage to the bhendi crop. Diseases are a key limitation to lower the bhendi yields (Sastry and Singh, 1974). Among them, powdery mildew is one of the most destructive disease and cause 65% yield loss. The genera of different powdery mildew fungi, obligate biotrophic pathogens are causing infection more than 10,000 host plant species. To protect the crops from powdery mildew infection, fungicides are indiscriminately applied frequently with inadequate dosage which cause fungicide resistance against powdery mildew fungi. In addition to that the fungicide are causing harmful effect on biodiversity, natural ecosystem and possess the residual problem (Maurya *et al.*, 2013). Physical and biological approaches have been proposed to support and replace chemical management of powdery mildews. Mycoparasites (fungi that parasitize other fungi) are abundant in the majority of terrestrial environments, with the most well-known species attacking fungal plant diseases (Kiss 2003). Numerous mycoparasites have been investigated extensively and economically used as crop disease biocontrol agents (BCAs) (Viterbo and Horwitz 2010). *A. quisqualis* is a unique mycoparasite of Erysiphales (Angeli *et al.*, 2013), and it is classified as an endoparasitic fungus because its conidia (pycnidiospores or spores, as some authors refer to them) penetrate into *Erysiphe cichoracearum* and generate pycnidia inside powdery mildew structures (Romero *et al.*, 2003). Molecular studies have shown that the mycoparasities are genetically diverse (Szentivanyi *et al.*, 2005). All of these studies were analysed on the basis of the nuclear ribosomal DNA gene internal transcribed spacer (ITS) region (rDNA). *Ampelomyces* spp. have a phylogenetic position in the order Dothideales, according to Kiss *et al.*, 1998. In the Dothideales, *Ampelomyces* ITS sequences and other ascomygetous fungus established two lineages (Arenal *et al*., 2000). The primary goal of this research was to determine the genetic variability in twenty isolates of *Ampelomyces* spp. from different ecosystems were evaluated with ten different RAPD markers.

MATERIALS AND METHODS

Isolation of mycoparasite. *Ampelomyces* isolates were isolated from powdery mildew-infested plants collected around Tamil Nadu. Effective 20 *Ampelomyces* isolates (Table 1) were selected based on mycoparasitic and plant growth promotion activity. All the cultures of ten isolates were maintained on liquid culture media (Potato Dextrose broth, PDB) for 7 days at 28±1ºC in a shaker and its DNA analyzed for genetic variability.

Table 1: Primers used for RAPD analysis.

Sr. No.	Primers	Sequence5'- 3'	PCR conditions			References
1.	OPA-01	CAGGCCCTTC	Initial denaturation	94°C for 10 min;	30 cycles	Bhat and Jarret (1995)
2.	OPA-03	AGTCAGCCAC	Denaturation	94ºC for 1min		
3.	OPA-05	AGGGGTCTTG	Annealing	36ºC for 1 min		
4.	OPA-07	GAAACGGGTG	Extension	72ºCfor2min		
5.	OPA-09	GGGTAACGCC	Final extension	72°C for 5 min.		
6.	OPA-18	AGGTGACCGT				
7.	OPB-02	TGATCCCTGG	Initial denaturation	94°C for 4 min,	35 Cycles	Franzin *et al*. (2004)
			Denaturation	94°C for 30 s		
			Annealing	35°C for 1 min,		
			Extension	72°C for 2 min		
			Final extension	10 min at 72°C.		
8.	OPC-08	TGGACCGGTG	Initial denaturation	94°C for 5 min;	40Cycles	Shinde *et al*. (2007)
9.	OPC-12	TGTCATCCCC	Denaturation	94ºC for 1min		
10.	OPE-01	CCCAAGGTCC	Annealing	36ºC for 1 min		
			Extension	72ºCfor 2min		
			Final extension	72°C for 5 min.		

Genetic analysis

DNA extraction. Pure cultures of the *Ampelomyces* isolates were maintained on PDA slants and incubated at 25±1°C for 7 days under controlled temperature. Mycelia were aseptically transferred to flasks of potato-dextrose broth (PDB, HI Media) and incubated for 5 days at 25±1°C. The mycelia were filtered from the liquid medium for the extraction of DNA. The CTAB method (Moller *et al*. 1992) was used to extract genomic DNA (gDNA) from each isolate, which was then measured using a Nano Drop 1000 spectrophotometer (Thermo Scientific). *Ampelomyces* cultures were identified molecularly using the conserved ribosomal internal transcribed spacer (ITS) region. Using the universal primer pairs ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), we amplified the ITS regions between the small nuclear 18S rDNA and the large nuclear 28S rDNA, including 5.8S rDNA. Amplification was carried out on a Thermal Cycler (Applied Biosystems 9700) using 25 µl reaction mixtures comprising 2.5 µl of 10X buffer (10 mMTris–HCl, pH 8.8); 2.5 mM MgCl2; 2 mM each dNTP; 25 pmolmM primer (ITS-1 and ITS-4); 1U Taq DNA Polymerase; and 60–100 ng genomic DNA. The amplification cycle initial denaturation at 95 °C for 10 min followed by 35 cycles at 94 °C for 30 s, 60 °C for 45sec and 72°C for 1 min and a final extension at 72°C for 8 min. Separation of amplified PCR products on an agarose gel (1.5 percent w/v) in 1X TAE buffer at 75 V for 90 minutes.

Table 2: Details of powdery mildew mycoparasitic isolates of *Ampelomyces quisqualis* isolated from different hosts.

Sr. No	Isolates	Plant host	*A. quisqualis* parasitic on powdery mildew pathogens	Geographical distribution
1.	AQ-TNAU-DST-01	*Abelmoschus esculentus* L.	*Erysiphe cichoracearum* DC.	Coimbatore
2.	AQ-TNAU-DST-02	*Vigna unguiculata* L.	*Erysiphe polygoni* DC.	Coimbatore
3.	AQ-TNAU-DST-03	*Sesamum indicum* L.	*Oidium erysiphoides*	Veppanthattai
4.	AQ-TNAU-DST-04	*Abelmoschus esculentus* L.	*Erysiphe cichoracearum* DC.	Kambam
5.	AQ-TNAU-DST-05	*Capsicum annuum* L.	*Leveillula taurica* (Lev.)	Krishnagiri
6.	AQ-TNAU-DST-06	*Abelmoschus esculentus* L.	*Erysiphe cichoracearum* DC.	Coimbatore
7.	AQ-TNAU-DST-07	*Vitis vinifera L.*	*E. necator* Schwein.	Dindugal
8.	AQ-TNAU-DST-08	*Tagetes erecta* L.	*Leveillula taurica* (Lev.)	Coimbatore
9.	AQ-TNAU-DST-09	*Capsicum annuum* L.	*Leveillula taurica* (Lev.)	Coimbatore
10.	AQ-TNAU-DST-10	*Abelmoschus esculentus* L.	*Erysiphe cichoracearum* DC.	Salem
11.	AQ-TNAU-DST-11	*Vitis vinifera L.*	*E. necator* Schwein.	Coimbatore
12.	AQ-TNAU-DST-12	*Tagetes erecta* L.	*Leveillula taurica* (Lev.)	Coimbatore
13.	AQ-TNAU-DST-13	*Abelmoschus esculentus* L.	*Erysiphe cichoracearum* DC.	Erode
14.	AQ-TNAU-DST-14	*Vigna unguiculata* L.	*Erysiphe polygoni* DC.	Dharmapuri
15.	AQ-TNAU-DST-15	*Sesamum indicum* L.	*Oidium erysiphoides*	Coimbatore
16.	AQ-TNAU-DST-16	*Abelmoschus esculentus* L.	*Erysiphe cichoracearum*	Krishnagiri
17.	AQ-TNAU-DST-17	*Vitisvinifera* L.	*E. necator* Schwein.	Coimbatore
18.	AQ-TNAU-DST-18	*Parthenium hysteroporus*	*Oidium parthenii*	Coimbatore
19.	AQ-TNAU-DST-19	*Vigna unguiculata* L.	*Erysiphe polygoni* DC	Tirupur
20.	AQ-TNAU-DST-20	*Cucumis sativus*	*Erysiphe cichoracearum*	Krishnagiri

Studying the genetic variability among the isolates of *A. quisqualis* using RAPD marker. Ten RAPD primers were used to determine the genetic diversity of twenty *A. quisqualis* isolates (Tables 1 and 2). DNA 25 ng/l, 10X buffer (containing 2.5 mmol l^{-1} $MgCl_2$) - 2 µl, 2 mmol l^{-1}d NTP mixture - 2µl, 2 mol l^{-1} primer - 5 µl, Taq DNA polymerase 3U and sterile water - 8 l make up the PCR reaction. PCR amplification was performed

Cluster analysis. The genetic similarity of twenty *Ampelomyces* isolates was assessed, based on RAPD data, using S17 Bray-Curtis similarity (Jaccard 1908). Based on RAPD data, the genetic similarity of twenty Ampelomyces isolates was analysed using S17 Bray-Curtis similarity (Jaccard 1908). The unweighted pair group technique of arithmetical averages (UPGMA) algorithm was then used to create a dendrogram from the data. The presence/absence of bands in fingerprint profiles was analysed using the PRIMER 7 statistical software (Plymouth Routines in Multivariate Ecological Research, version 7.0.9; PRIMER-E, Plymouth, UK). The Bray–Curtis coefficient was used to calculate similarities between each pair of samples, resulting in a similarity matrix (Clarke, 1993). The similarity data was ordinated using non-metric multi-dimensional scaling (MDS). MDS ordination produces a map in which each sample's position is decided by its distance from all other points in the analysis. The plot's stress indicates how much distortion was created to allow the data to be represented in the specified dimensions. A stress value of more than 0.2 implies a plot that is close to random, less than 0.2 offers a useful two-dimensional representation, and less than 0.1 represents an ideal ordination with no significant risk of misinterpretation (Clarke, 1993). Within PRIMER 7, stress was calculated as stated by Kruskal (1964). The similarity matrix using the Bray– Curtis coefficient was also analysed by Hierarchical Cluster Analysis (HCA), a classification method that aims to group discrete clusters based on similarity, to visualise the relationship between different isolates of *A. quisqualis* using RAPD. Using PRIMER 7 software, a dendrogram was generated from the prioritised similarities using a weighted, group average linkage agglomerative technique.

The effectiveness of RAPD molecular markers on assessing the genetic variability of isolates of *E. cichoracearum*

The analysis included bands that were common to all isolates. By counting the number of bands formed by each primer in all twenty isolates, the characteristics of banding pattern, such as total number of bands, number of monomorphic, polymorphic bands per primer, and percent polymorphism, were calculated. In addition, the RAPD and ISSR markers assay efficiency index (AEI = Polymorphic bands/Total number of primers) was recorded.

Anderson *et al.* 1993 devised a method to quantify polymorphism information content (PIC). Each locus' PIC value was estimated as follows:

$$PIC_j = 1 - \sum_{l=1 \text{ to } L} P^2_{lj}$$

P_{lj} is the relative frequency of the l^{th} allele for the locus j, and it is averaged across all alleles (L) throughout all lines. The PIC calculates a locus discriminating power by taking into consideration not just the number of alleles expressed, but also the relative frequencies of those alleles. With numerous loci in equal frequency, PIC values might vary from 0 (monomorphic) to 1 (very discriminative).

$$EMR\ (E) = n_p\ (n_p/n)$$

where n_p denotes polymorphic loci and n denotes the total number of loci (Sornakili *et al.*, 2017).

The Marker Index (MI) was calculated by multiplying the PIC by the number of polymorphic bands per assay unit, and the Effective Multiplex Ratio (EMR) was calculated by multiplying the proportion of polymorphic loci by the number of polymorphic loci for each assay

RESULTS AND DISCUSSION

The present study is the first extensive investigation of quantitative aspects of a mycoparasite *A. quisqualis* in different host range from different districts of Tamil Nadu.

Genetic variability analysis of isolates of *A. quisqualis* by RAPD marker. DNA markers have turned into a very useful tool for studying the taxonomy of species. The random amplified polymorphic DNA (RAPD) approach, which is based on the polymerase chain reaction (PCR), is one of the most frequently used molecular techniques for the development of DNA markers (Levi *et al*., 2001). It enables rapid assessment of genetic variability across taxonomic groups and has been used to investigate inter- and intraspecific variability among isolates of many fungal species utilized in biological control (Tigano*et al*., 1995). In this present study, RAPD analysis was performed on twenty isolates of *A. quisqualis*was amplified using 10 RAPD primers. The ten primers amplified products ranging in size from 0.2 to 1.5 kb. The vast majority of ten RAPD primers showed a moderate level of polymorphism. Three primers *viz.*, OPA5, OPA7 and OPF6 produced fragment profiles showing at least one amplicon common to all isolates of *A. quisqualis* (Fig. 2).

Two-dimensional Non-metric Multi-Dimensional Scaling (MDS) plot using the Bray-Curtis similarity index ordinating the samples using the data from RAPD discriminated *A. quisqualis* from each isolates (Fig. 2a). The different isolates of *A. quisqualis* were evenly distributed in the MDS plot with less Kruskal's stress (0.09). The isolates *A. quisqualis* AQTNAU-DST16, 18 and 20 are slackly different placed in the MDS plot, which shared different ordination. Six isolates of *A. quisqualis viz.*, AQTNAU-DST10 to 15 shared the same similarity of coordination in the plot.

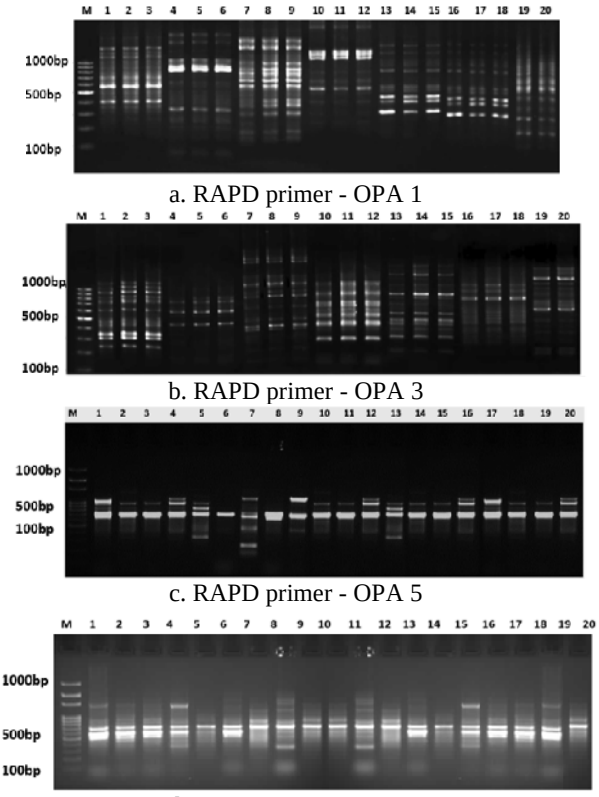

Fig. 1. Polymorphism resultant for *Ampelomyces quisqualis* isolates M-Marker, 1)AQTNAU-DST1, 2) AQTNAU-DST2, 3) AQTNAU-DST3, 4) AQTNAU-DST4, 5) AQTNAU-DST5, 6) AQTNAU-DST6, 7) AQTNAU-DST7, 8) AQTNAU-DST8, 9) AQTNAU-DST9, 10) AQTNAU-DST10,11)AQTNAU-DST11, 12)AQTNAU-DST12, 13) AQTNAU-DST13,14) AQTNAU-DST14,15) AQTNAU-DST15, 16)AQTNAU-DST16, 17) AQTNAU-DST17, 18) AQTNAU-DST18, 19) AQTNAU-DST19,20) AQTNAU-DST20.

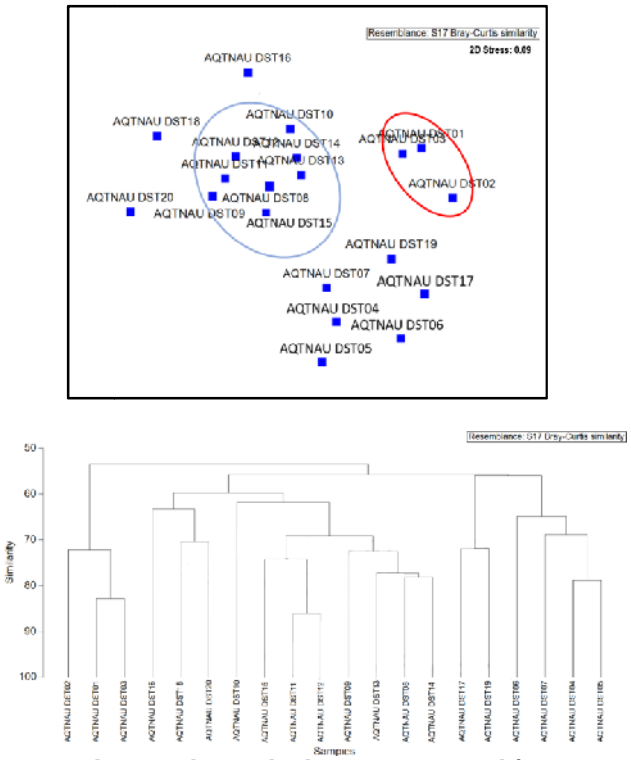

Fig. 2. (a) Non-metric MDS plot (a) and HCA dendrogram constructed from RAPD fingerprint data of *Ampelomyces quisqualis* isolates. The similarity matrix using the Bray-Curtis coefficient was applied for both the analyses in Primer 7.0 software.

This results commensurate with Rajalakshmi *et al.*, (2016) described the (MDS) plot using the Bray-Curtis similarity index for different isolates of *C. globosum* were with Kruskal's stress values of 0.07. The isolates of *A. quisqualis* (AQTNAU-DST04-07, AQTNAU-DST17 and AQTNAU-DST19) show separate cluster similarity of 58%. Isolates of AQTNAU-DST11 and AQTNAU-DST12 shows 86% similarity followed by AQTNAU-DST01 and AQTNAU-DST03 showed 83% similarity cluster. Genetic diversity using AFLP markers in this fungus was also explored earlier (Aggarwal et al., 2003), showing that five *C. globosum* isolates formed two distinct clusters, one comprising isolates Cg6, Cg7 and Cg8, and the other encompassing isolates Cg1 and Cg5.

To depict genetic relationships in the form of a dendrogram, a genetic similarity estimate (S17 Bray Curtis coefficient) based on RAPD banding pattern was performed for cluster analysis. In this Dendrogram, HCA-based clustering of isolates showed 55-85 % similarity. The studied isolates were clearly classified into two groups based on the dendrogram. Cluster A, the first group, consisted of three isolates: AQTNAU-DST01, AQTNAU-DST02, and AQTNAU-DST03. Cluster B, which contained two sub clusters including AQTNAU-DST08 to AQTNAU-DST16, 18 and 20were clubbed and formed sub cluster 1 whereas AQTNAU DST 04 – 07, 17 and 19 clustered together to form sub cluster 2. The present study's findings corroborate those of Delye *et al*., (1997), who described the diversification of *Ampelomyces.* The RAPD markers were able to distinguish all isolates at 94% similarity level. Similar results were also obtained in previous researchers (Zimand *et al*., 1994; Hermosa *et al*., 2001; Grendene *et al*., 2002).

Effectiveness of RAPD marker in assessing the genetic variability of *A. quisqualis* isolates. PIC scores of each primer ranged from 0.56 (OPA-05) to 0.91 (OPG-11), with an average of 0.67. The EMR value ranged from 3.20 (OPA-05, OPA-07 and OPA-09) to 7.11 (OPG-05), with an average of 4.36 indicating hyper variability among the individuals studied. To determine the overall usefulness of a given marker system, the marker index (MI) was calculated for RAPD with an average value of 2.95 was determined per primer (Table 3). The estimates of MI were found to be highest for primer OPG-05 (5.05) and the lowest with the primer OPA-05 (1.79). Singh and Sengar *et al.*, 2015 showed that the analysis of RAPD indicated RAPD gave maximum PIC values of 0.9925. RAPD primer OPF-13 gave the maximum accessions coverage depending on the value of PIC in the rice genome. Rakhonde *et al*. (2015) reported that genetic variability (RAPD primers) among eighteen Indian isolates of *Fusarium oxysporum* f. sp. *ciceri* causing wilt in chickpea. The PIC value ranged from 0.504 to 0.926 across eighteen isolates of *Fusarium oxysporum* f. sp. *ciceri* indicating high degree of variation in respect to genetic similarity. This findings are in accordance with El Komy *et al*., (2015) studied the genetic diversity of *Trichoderma* sp by RAPD fingerprinting and the data exhibited a wide range of genetic similarity coefficients (0.443 to 0.944) with an average of 0.76.

Table 3: Genetic variability of the isolates of *A. quisqualis* revealed by RAPD analysis.

Sr. No.	Primers	Total no. of bandingpattern	No. of polymorphic banding pattern	No. of monomorphic banding pattern	PIC Value	EMR value	MI
1.	OPA-01	6	5	1	0.65	4.17	2.71
2.	OPA-03	6	5	1	0.71	4.17	2.96
3.	OPA-05	5	4	1	0.56	3.20	1.79
4.	OPA-07	5	4	1	0.63	3.20	2.02
5.	OPA-09	5	4	1	0.64	3.20	2.05
6.	OPF-06	6	5	1	0.55	4.17	2.29
7.	OPF-10	7	6	1	0.67	5.14	3.44
8.	OPG-05	9	8	1	0.71	7.11	5.05
9.	OPG-08	7	6	1	0.67	5.14	3.44
10.	OPG-11	4	4	1	0.91	4.17	3.79
Total	61	51	10	0.67	4.36	7.94	2.95

PIC-Polymorphic Information Content; EMR- Effective Multiplication Ratio
MI – Marker Index

CONCLUSION

RAPD primers were used to evaluate the genetic diversity of twenty *Ampelomyces* mycoparasite isolates from diverse powdery mildew hosts in Tamil Nadu. The genetic similarity coefficients showed that any two or more isolates were identical. Twenty isolates had up to 90% genetic diversity, according to RAPD profiles. The results of this study show that the isolates obtained from various geographical areas have a significant level of genetic diversity. The RAPD profile revealed regional variation due to diverse powdery mildew hosts.

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REFERENCES

Angeli, D. (2013). New insights into host specialization and mycoparasitic interaction between powdery mildew fungi and *Ampelomyces quisqualis*.

Arenal, F., Platas, G., Monte, E., & Peláez, F. (2000). ITS sequencing support for *Epicoccumnigrum* and *Phomaepicoccina* being the same biological species. *Mycological Research, 104*(3): 301-303.

Benchasri, S. (2012). Okra (*Abelmoschus esculentus* (L.) Moench) as a valuable vegetable of the world. *Ratarstvoipovrtarstvo, 49*(1): 105-112.

Bhat, K. V., & Jarret, R. L. (1995). Random amplified polymorphic DNA and genetic diversity in Indian Musagermplasm. *Genetic Resources and Crop Evolution, 42*(2): 107-118.

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, *18*(1): 117- 143.

Delye, C., Laigret, F., & Corio-Costet, M. F. (1997). A mutation in the 14 alpha-demethylase gene of *Uncinulanecator* that correlates with resistance to a sterol biosynthesis inhibitor. *Applied and Environmental Microbiology*, *63*(8): 2966-2970.

Gaitán- Solís, E., Duque, M. C., Edwards, K. J., & Tohme, J. (2002). Microsatellite Repeats in Common Bean (*Phaseolus vulgaris*) Isolation, Characterization, and Cross-Species Amplification in *Phaseolus* ssp. *Crop science*, *42*(6): 2128-2136.

Grendene, A., Minardi, P., Giacomini, A., Squartini, A., & Marciano, P. (2002). Characterization of the mycoparasite *Coniothyrium minitans*: comparison between morpho-physiological and molecular analyses. *Mycological Research*, *106*(7): 796-807.

El Komy, M. H., Saleh, A. A., Eranthodi, A., & Molan, Y. Y. (2015). Characterization of novel *Trichoderma asperellum* isolates to select effective biocontrol agents against tomato Fusarium wilt. *The Plant Pathology Journal*, *31*(1), 50.

Hermosa, M. R., Grondona, I., Díaz-Mínguez, J. M., Iturriaga, E. A., & Monte, E. (2001). Development of a strain-specific SCAR marker for the detection of *Trichoderma atroviride* 11, a biological control agent against soil borne fungal plant pathogens. *Current Genetics*, *38*(6): 343-350.

Jaccard, P. (1908). Nouvellesrecherchessur la distribution florale. *Bull. Soc. Vaud. Sci. Nat.*, *44*: 223-270.

Kruskal, J. (1964). Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika*, *29*(1): 1-27.

Kiss, L. (1998). Natural occurrence of *Ampelomyces* intracellular mycoparasites in mycelia of powdery mildew fungi. *New Phytologist*, *140*(4): 709-714.

Kiss, L. (2003). A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. *Pest Management Science: formerly Pesticide Science*, *59*(4), 475-483.

Franzin, L., &Cabodi, D. (2004). Molecular typing of *Yersinia* strains by pulsed-field gel electrophoresis and RAPD-PCR. In *The Genus Yersinia* (pp. 349-352).

Levi, A., Thomas, C.E., Keinath, A.P. and Wehner, T.C., 2001. Genetic diversity among watermelon (*Citrullus lanatus* and *Citrullus colocynthis*) accessions. *Genetic Resources and Crop Evolution*, *48*(6), pp.559-566.

Maurya, R. P., Bailey, J. A., & Chandler, J. S. A. (2013). Impact of plant spacing and picking interval on the growth, fruit quality and yield of okra (*Abelmoschus esculentus* (L.) Moench). *American Journal of Agriculture and forestry*, *1*(4), 48-54.

Möller, E. M., Bahnweg, G., Sandermann, H., & Geiger, H. H. (1992). A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic acids research*, *20*(22): 6115.

Németh, M. Z., Mizuno, Y., Kobayashi, H., Seress, D., Shishido, N., Kimura, Y., & Nonomura, T. (2021). *Ampelomyces* strains isolated from diverse powdery mildew hosts in Japan: their phylogeny and mycoparasitic activity, including timing and quantifying mycoparasitism of *Pseudoidium neolycopersici* on tomato. *PloS one*, *16*(5): e0251444.

Rajalakshmi, J. R., Devi, D. D., Harish, S., Raguchander, T., & Harish, S. (2016). Genomic differentia genomic differentiation among *Chaetomium globosum* Isolates by using RAPD and ISSR Markers. *The bioscan*, *11*(3):1941-1945.

Rakhonde, P. N., Mane, S. S., Gawande, A. D., Bangar, S. S., &Moharil, M. P. (2015). Molecular and pathogenic variability among Indian isolates of *Fusarium oxysporum* f. sp. *ciceri* causing wilt in Chickpea. *The Ecoscan*, *7*: 21-28.

Romero, D., Rivera, M. E., Cazorla, F. M., De Vicente, A., & Perez-Garcia, A. (2003). Effect of mycoparasitic fungi on the development of *Sphaerotheca fusca* in melon leaves. *Mycological research*, *107*(1): 64-71.

Sastry, K.S.M. and S.J. Singh, 1974. Effect of yellow vein mosaic virus infection on growth and yield of okra crop. *Indian Phytopath*, *27*: 294–297.

Sawant, I. S., Wadkar, P. N., Ghule, S. B., Salunkhe, V. P., Chavan, V., & Sawant, S. D. (2020). Induction of systemic resistance in grapevines against powdery mildew by *Trichoderma asperelloides*strains. *Australasian Plant Pathology*, *49*(2): 107-117.

Singh, A., & Sengar, R. S. (2015). DNA fingerprinting based decoding of indica rice (Oryza sativa L) via molecular marker (SSR, ISSR, & RAPD) in aerobic condition. *Adv. Crop. Sci. Technol.*, *3*, 167.

Shinde, M., Dhalwal, K., Mahadik, K. R., Joshi, K. S. & Patwardhan, B. K. (2007). RAPD analysis for determination of components in herbal medicine. *Evidence-based Complementary and Alternative Medicine*, *4*(1): 21-23.

Szentiványi, O., Kiss, L., Russell, J. C., Kovács, G. M., Varga, K., Jankovics, T., & Jeffries, P. (2005). *Ampelomyces* mycoparasites from apple powdery mildew identified as a distinct group based on single-stranded conformation polymorphism analysis of the rDNA ITS region. *Mycological Research*, *109*(4): 429-438.

Tigano-Milani, M.S., Honeycutt, R. J., Lacey, L. A., Assis, R., McClelland, M. & Sobral, B. W. 1995. Genetic variability of *Paecilomyces fumosoroseus* isolates revealed by molecular markers. *Journal of Invertebrate Pathology*, *65*(3): 274-282.

Viterbo, A., & Horwitz, B. A. (2010). Mycoparasitism. *Cellular and molecular biology of filamentous fungi*, 676-693.

Zimand, G., Valinsky, L., Elad, Y., Chet, I., & Manulis, S. (1994). Use of the RAPD procedure for the identification of *Trichoderma* strains. *Mycological Research*, *98*(5): 531-534.

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