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Isolation and Identification of Storage Fungi from Citrus Sampled from Major Growing areas of Punjab, Pakistan

Nosheen Akhtar[1], Tehmina Anjum[1]* and Rasheda Jabeen[1]
[1]*Institute of Agricultural Sciences, Quaid-e-Azam Campus, University of the Punjab, Lahore-54590, Pakistan*
*For Correspondence: tehminaanjum@yahoo.com

Abstract

Stored citrus fruits were collected from cold storage houses of major citrus growing areas of the Punjab, Pakistan and storage fungi were isolated from the sampled fruits. The fungal isolates were identified as *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. terreus*, *Penicillium verrucosum*, *Rhizopus arrhizus*, *R. stolonifer*, *A. parasiticum, Fusarium oxysporum*, *P. citrinum*, *A. awamorii*, *Alternaria alternata*, *F. solani* and *Mucor* sp. based on morphological characteristics. The frequency of occurrence of each fungal species was determined; *P. italicum* was found the most frequent with the highest occurrence of 29.75% among all isolates, followed by the *A. niger* (14.87%), while *Dreshlera* sp. having the least occurrence (0.82%). Hence, *P. italicum* was chosen for the molecular characterization among its different geographically distinct isolates through random amplified polymorphic DNA (RAPD) technique. The UPGMA based dendrogram showed three distinct groups, which coincide with the geographical locations from where the isolates were collected. The total variation of 23% among the isolates confirmed that the isolates are of the same species and the source of variation can be due to the environmental factors. The variation can also be contributed by switching off and switching on of some genes under certain environmental conditions.

Keywords: Citrus; Fungi; *Penicillium italicum*; RAPD; Random primers

Introduction

Citrus is one of the most important winter fruit crops of the world and has grown commercially in more than 100 countries across six continents (Terol *et al.,* 2007). They contain several phytochemicals including vitamin C with disease preventing and life sustaining functions (Dillard and German, 2000). Pakistan is ranked 10th in citrus production worldwide (Sabir *et al.,* 2010). Moreover, huge economic losses occur throughout the world due to post harvest diseases. In Pakistan, about 40% of total citrus produced is wasted during storage in post-harvest process (Naseer, 2010), and is frequently reduced by fungal pathogens attack (Liu *et al.,* 2007). Storage fungi can reduce the shelf life and acceptability of fresh produce.

Blue mold, caused by *Penicillium italicum,* is generally severe postharvest disease of citrus, which costs the loss up to 25% of the total production worldwide (Palou *et al.,* 2007; Montesinos-Herrero *et al.,* 2009). Mold growth in citrus fruit leads to the production of the hazardous mycotoxins (Moss, 2008). Taxonomic relationships of fungal species have been clarified by applying many phenotypic and genotypic approaches (Varga *et al.,* 2000).

The molecular markers including biochemical protein markers and DNA markers are extremely valuable tools for weighing genetic similarity and determining species individualities. Among the DNA molecular markers, random amplified polymorphic DNA (RAPD) typically are more eminent due to high variability and reproducibility with an added advantage of no prior knowledge of DNA sequence for the fingerprinting of any genomic DNA. Analysis of genetic diversity among closely related species is crucial step towards understanding the fungal populations.

The objectives of this investigation were to characterize storage fungi isolated from stored citrus fruit of different storage houses in Punjab using morphological characteristics and to find the most occurring storage fungi among the storage fungi and to assess the diversity among these isolates through RAPD-PCR technique to show genetic variability within and between these isolates.

Materials and Methods

Sample Collection

Samples of citrus fruits were collected from different cold storage houses in eight major citrus growing areas of Province Punjab, Pakistan. These samples were placed in separate sterile plastic bags; transferred to the laboratory and kept in refrigerator at 4^{o}C till further analysis. These regions were selected on the basis of their fruit production importance.

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Isolation and Identification of Storage Fungi

Isolation of storage fungi from each of the collected fruit sample was carried out by using the technique of Baiyewu *et al.* (2007). A small portion of disease tissue were cut with sterile scalpel and placed on previously prepared Malt extract agar and Potato dextrose agar medium plates and incubated at 25±1°C for 7 days. The developing fungal colonies were counted to calculate percentage frequency and further characterized on morphological basis by using the most documented keys and literature for fungal identification (Samson and Varga, 2007). Percentage frequency of individual isolated fungal species was determined by using the method described by Giridher and Ready (1997) as:

$$\text{Frequency (\%)} = \frac{\text{No. of observations in which species appeared}}{\text{Total no. of observations}} \times 100$$

Finding Genetic variation in all Isolates

According to the percentage of occurrence, *P. italicum* was selected for genetic analysis as a most abundant blue mold fungus on citrus during storage. Genetic variation of eight morphologically similar but geographically distinct *P. italicum* isolates was carried out using RAPD analysis.

DNA Extraction

The total genomic DNA was extracted by using CTAB method (Doyle and Doyle, 1991) form the fungal mat and ground to fine powder with Fermentas glass beads. The freshly prepared pre-warmed (65ºC) extraction solution was added to the powdered fungal mass in new autoclaved Eppendorf (1.5 mL) tubes and incubated at 65ºC. An equal volume of solution Choloform isoamyl alcohol (24:1) was added and centrifuged at 10,000 × *g* to separate the phases. Aqueous supernatants were transferred to new tubes and 2⁄3 volume of cold isopropanol was added. The sample was centrifuged and transparent DNA pellets were obtained. After washing, the pellets were air dried and resuspended in 50 µL of Tris HCl EDTA (TE) buffer.

Estimation of Quantity and Quality of DNA

The quantity and quality of extracted DNA was determined by Techne Spec gene Spectrophotometer (140801-2, UK). The concentration was calculated on assumption that absorbance of 1 at 260 nm is equivalent to 50 mg⁄mL double stranded DNA or 40 mg⁄mL single stranded DNA (Sambrook *et al.,* 1989). The quantity of DNA was calculated by the following equation:

$$\text{DNA Con (µg/mL)} = \text{Absorbance at 260 nm} \times \text{Dilution factor} \times 50$$

The quality of the DNA was also estimated through 1% agarose gel electrophoresis in which DNA bands were compared with the 1kb DNA markers showing the concentrations of DNA in ng/µL. Hence, the DNA concentration was maintained to 25 ng/µL for polymerase chain reaction (PCR) amplifications.

Random Amplification of Polymorphic DNA Analysis

The RAPD analysis is a valuable tool for studying DNA polymorphism in different fungal isolates for exploring phylogenetic relationships among them. RAPD analysis was carried out by following the method described by Ranganath *et al.* (2002).

Random Primer Screening

A set of 20 primers procured from School of Biological Sciences (SBS) Genetech Co. Ltd-Beijing, China were used in RAPD analysis for the initial screening (Table 1). Majority of these primers produced clear, distinct and reproducible polymorphic bands in different isolates of *P. italicum*. The primers were diluted up to 100 picomole concentration before use in RAPD analysis. PCR reactions were carried out in 25 µL volume containing PCR Buffer (10X), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, and 0.6 U DNA polymerase (Enzynomics, Korea). PCR conditions and separation of RAPD-PCR fragments were done according to Messner *et al.* (1994). The amplifications were carried out in Techne-412 thermal cycler with temperature profile as initial denaturation at 94ºC for 5 min and then primers were subjected for denaturation at 94ºC for 1 min, annealing at 25ºC for 1 min and final extension at 72ºC for 5 min to a total of 40 cycles.

Statistical Analysis

RAPD profiles were recorded by visually comparing RAPD amplification profiles and scoring the presence or absence of each band for each primer (Halmschlager *et al.,* 1994). The bands obtained from 2% agarose gel electrophoresis were combined in a binary matrix in two - discrete - characters - matrix (0 and 1 for absence and presence of RAPD – markers, respectively). In order to assess over all distribution of genetic diversity, data was analyzed by using MINITAB software (MINITAB, 2004).

Results

In the present study, different fungi associated with the deterioration of citrus fruit during cold storage were isolated from the collected fruit samples. A total of sixteen fungal species belonging to seven different genera included were isolated and identified on the basis of their cultural and morphological characteristics which are revealed in detail in Table 1. The frequency of occurrence of each isolate of fungi which showed that *P. italicum* was the most frequently isolated fungus with the highest occurrence of 29.75% among all isolates, followed by the *Aspergillus niger* (14.87%), *A. flavus* (9.09%), *A. fumigatus* (7.43%), *A. terreus* and *P. verrucosum* (5.78%), *Rhizo pus arrhizus* (4.95%), *R. stolonifer* (4.13%), *A. parasiticus* and *Fusarium*

Table 1: Morphological Characteristics of Different Storage Fungi Isolated from Stored Citrus Fruit

Name of fungus	Colony diameter	Colony Texture	Obverse	Reverse	Character of hyphae	conidiophore	conidia
Penicillium italicum	5-6 cm		bluish green	yellow brown	septate, hyaline	smooth walled	ellipsoidal to cylindrical
Penicillium verrucosum	2.5-3.5 cm	velvety or almost floccose	grey green	yellowish brown	septate, hyaline	two-stage branched, rough walled	globose to subglobose
Penicillium citrinum	2-3 cm		blue green	bright yellow	septate, hyaline	smooth walled, matulae and phailides present	globose to subglobose, sooth walled
Aspergillus niger	5-6 cm	powdery	black	Off-white	conidial head radiate, conidia matulae and phailides present	broad, long, thick walled, brownish	globose , irregularly roughened,
Aspergillus flavus	6-7 cm	powdery	yellow green	Pale brown	conidial head radiate,	hyaline, long, rough walled,	globose to subglobose, finely roughened to echinulate
Aspergillus fumigatus	7 cm		bluish green	Greyish	Vesicle pyriform, uniseriate	clavate vesicle, thick walled	Smooth, globose to subglobose
Aspergillus terreus	6-7 cm	velvety or almost floccose	orange brown to brown	uncolored	matulae present, conidial heads columnar,	smooth walled, hyaline, with hemispherical vesicle	globose to slightly ellipsoidal, smooth walled
Aspergillus awamorii	5-6cm		green	green	matulae and phailides present	smooth walled, vesicle subglobose	cylindrical to ellipsoidal, coarsely roughened
Aspergillus parasiticus	5-6 cm		green	colorless	Uniseriate, vesicle spherical	roughened, matulae absent	echinulate and coarsely spiny
Rhizopus arrhizus		cottony	white	brown	Hyphae branched and aseptate, rhizoids present	sporangiophore short, columella globose	spoangiospores ovoid to more or less globose, dark in color
Rhizopus stolonifer		fluffy	greyish	yellow brown	columella subglobose to oval	sporangiophore subglobose rigid,	sporangia pale to brown, straight, sporangiospore thick walled
Mucor sp.	full plate	cottony	white to yellow	Brownish	Columella obovoid, ellipsoidal, with truncate base	Sporangiophore short branches, recurved and encrusted wall	Sporangia brownish to grey, spores ellipsoidal to subglobose
Fusarium oxysporum	4.6-6.5 cm	sparce to floccose	white	purple	Macroconidia present		septate, fusiform, moderately curved, pointed at both ends
Fusarium solani	4.5-6.5	floccose	whitish	cream	macroconidia present		curved, short, with blunt apical and pedicellate basal cells
Alternaria alternata	6-7 cm		dark brown blackish,		Hyphae branched and septate, brown in color	conidiophore simple, straight and curved	conidia formed in long chain, ovoid, obclavate and dark brown in color
Dreshlera sp.	4-5.5 cm		dark brown to black	yellow to brown	conidiophore , conidia	simple and branched	Cylindrical or ellipsoidal, septate with round ends.

oxysporum (3.3%), *P. citrinum, A. awamorii* and *Alternaria alternata* (2.47%), *F. solani* and *Mucor* sp. (1.65%) with *Dreshlera* sp. having the least occurrence of 0.82% (Table 2).

The morphological characteristics of *P. italicum* such as colonies on Malt Extract Agar (MEA) medium were 5-6 cm in diameter after 7 days. The colonies were bluish green in color with velvety and floccose and reverse side of the colony is yellow to brown. The microscopic characteristics such as septate and hyaline hyphae; conidiophores smooth walled and conidia ellipsoidal to cylindrical in shape smooth walled and blue in color were observed. The cultural and microscopic characteristics of *P. italicum* seen in the present study were correlated to those as described by Singh *et al.* (1991).

Eight geographically distinct isolates of *P. italicum* were collected during this study and subjected to further characterization by using RAPD-PCR technique (Table 3). A total of 20 RAPD decamers (Table 4) were used for testing the genetic variability among eight isolates of *P. italicum* of which two primers (Primer SBSA08 and SBSA15) did not show any amplification and hence dropped in the initial screening of the random primers. Remaining 18 random primers produced easily scorable and reproducible banding patterns, which were designated in genetic coefficient matrix in Minitab 16 software for further analysis. Total amplified polymorphic bands ranged from approximately 200 bp to 2000 bp sizes in the RAPD profile. There was a low percentage of polymorphism in different isolates of *P. italicum*. The most probable reason is that the isolates are of single species, which was correspondingly seen by their similar morphology. The average number of polymorphic bands perceived per primer was 8.22. The RAPD profiles produced with the primers (SBSA06, SBSA07, SBSA11, SBSA12, SBSA13, and SBSA18) and banding pattern are shown in Fig. 1. The ladder 100 bp was run as markers on

Table 2: Frequency of occurrence of fungi isolated from stored citrus fruit

Fungi isolated	% Frequency of occurrence
Penicillium italicum	29.75
P. verrucosum	5.78
P. citrinum	2.47
Aspergillus niger	14.87
A. flavus	9.09
A. fumigatus	7.43
A. terreus	5.78
A. awamorii	2.47
A. parasiticus	3.3
Rhizopus arrhizus	4.95
R. stolonifer	4.13
Mucor sp.	1.65
Fusarium oxysporum	3.3
F. solani	1.65
Alternaria alternata	2.47
Dreshlera sp.	0.82

Table 3: Qualitative and quantitative spectrophotometric analysis of extracted DNA

P. italicum isolates	Spectrophotometric reading of Extracted DNA		Ratio 260/280	Conc. of Extracted DNA (µg/ml)
	at 260 nm	at 280 nm		
Pi. Lhr	1.504	1.332	1.13	60
Pi Mul	0.812	0.866	0.94	29
Pi Fsd	0.046	0.056	0.82	1.0
Pi Chn	1.479	1.303	1.13	59
Pi Jhg	0.084	0.080	1.04	3.0
Pi Bhk	0.163	0.175	0.93	6.0
Pi Sh	0.001	0.001	1.00	4.5
Pi Sgd	0.123	0.117	1.06	5.0

both sides along with the negative control in which only water was used instead of DNA that indicates the credibility of the reaction mixture having no amplified band.

A dendogram based on UPGMA analysis indicated that the eight isolates formed three major groups GI, G2 and G3 confirming some level of genetic variation among the isolates of *P. italicum* (Fig. 2). The similarity coefficient ranged from 0 to 0.23 indicating that there is no 100% similarity occurs among any two isolates. Group 1 was further divided in to three subgroups containing five isolates naming Pi Lhr, Pi Mul, Pi Jhg, Pi Bhk and Pi Sdg. G2 consisted of two isolates, Pi Fsd and Pi Chn while G3 consisted of only one isolate, Pi Sh.

The first subgroup consisted of two isolates collected from Lahore and Multan, which were 3% distant from each other while this subgroup is 9% distant from the second subgroup containing two isolates collected from Jhang and Bhakar regions of Punjab. There is much similarity seen in isolates collected from Jhang and Bhakar, which are 1% distant from each other. This subgroup was 5% distant from the third subgroup which contained only one isolate collected from Sargodha cold storage house. G2 is 2% distant from G1, while G3 was 4% and 6% distant from G1 and G2, respectively.

Discussion

This study showed that a number of storage fungi of genera *Aspergillus, Penicillium, Rhizopus, Fusarium, Alternaria, Dreschlera*, and *Mucor* are associated with the spoilage and loss of citrus fruit during storage in Pakistan. The most abundant of all these fungi was *P. italicum*. Several *Penicillium* species were frequently established on citrus fruits and cause storage rot commonly referred as blue and green mold (Filtenborg *et al.*, 1996). Bukar *et al.* (2009) who reported that six genera of fungi namely *Penicillium sp., Aspergillus sp., Fusarium sp., Rhizopus sp., Alternaria sp.* and *Mucor sp.* were associated with massive deterioration of citrus fruit. Similarly, Niji *et al.* (1997) reported that *A. niger* was associated with the decay of citrus fruit. *P. digitatum, R. stolonifer* and *A. niger* have also

Table 4: Random twenty decamers used in initial screening, their accessions, sequences, number of polymorphic bands, percentage of polymorphic products and size of bands produced by each primer

Name	Sequence (5'- 3')	nmol	Total no. of Bands	No. of poly morphic bands	%age of polymorphic bands	Size of bands (bp)
SBSA01	CAG GCC CTT C	111.3	40	11	28%	250-1000
SBSA02	TGC CGA GCT G	108.4	39	7	17%	200-1500
SBSA03	AGT CAG CCA G	110.1	39	15	38%	200-700
SBSA04	AAT CGG GCT G	107.6	45	10	7%	300-1000
SBSA05	AGG GGT CTT G	106.5	29	5	17%	300-900
SBSA06	GGT CCC TGA C	109.9	37	5	14%	200-1200
SBSA07	GAA ACG GGT G	105.9	34	13	38%	400-1800
SBSA09	GGG TAA CGC C	108.1	46	6	13%	200-1400
SBSA10	GTG ATC GCA G	107.6	39	15	38%	270-1500
SBSA11	CAA TCG CCG T	110.4	41	9	22%	200-1600
SBSA12	TCG GCG ATA G	107.6	42	18	43%	400-1900
SBSA13	CAG CAC CCA C	112.2	63	7	11%	340-2000
SBSA14	TCT GTG CTG G	108.2	14	6	43%	350-800
SBSA16	AGC CAG CGA A	108.3	10	2	20%	300-1500
SBSA17	GAC CGC TTG T	109.3	11	3	27%	900-1500
SBSA18	ACG TGA CCG T	107.6	50	2	4%	200-1400
SBSA19	CAA ACG TCG G	108.7	47	7	15%	250-1100
SBSA20	GTT GCG ATC C	109.3	45	5	11%	200-1100

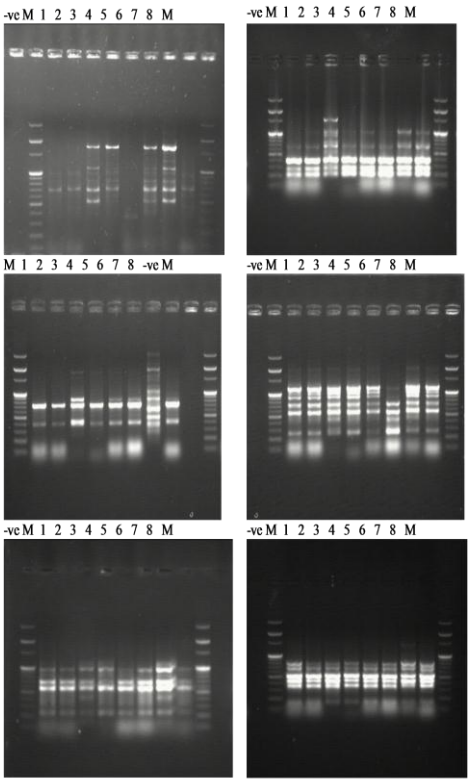

Fig. 1: RAPD profile of eight isolates (1-8 shown in Table 3) of *P. italicum* produced by random primers (SBSA06, SBSA07, SBSA11, SBSA12, SBSA13, and SBSA18. Molecular weight markers (M in bps) are indicated on the both sides (100 bp DNA ladder, cat # DM001), -ve sign shows the well with negative control in PCR reaction

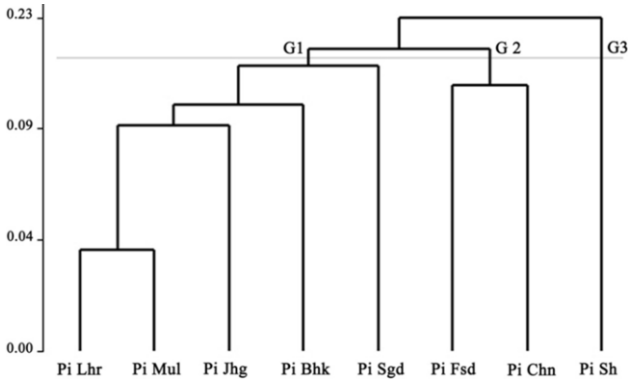

Fig. 2: UPGMA cluster analysis based dendogram depicting the genetic relationship among different *P. italicum* isolates

been isolated from orange fruits (Akintobi *et al.,* 2011). Al-Hindi *et al.* (2011), showed that more than 90% of citrus fruit were under fungal decay. Numbers of *Aspergillus* species have been reported such as A. *niger*, *A. fumigatus*, *A. nidulans*, *A. variecolour* and *A. candidus*. *Fusarium spp* were also the most commonly associated fungi in citrus fruits (Tournas and Katsoudas, 2005).

The morphological characteristics of *P. italicum* of the lab cultured isolates collected from different citrus producing districts of Punjab in Pakistan were closely related. This morphological study was not sufficient in examining the diversity among these isolates. Therefore, with the advent of modern approaches including molecular and genetic marker studies, these isolates were further characterized by the RAPD fingerprinting technique. Dupont *et al.* (1999) reported that the molecular markers reveal comparatively significant level of similarities among twelve *Penicillium* species whereas morphological characterization exhibited moderate level of similarities. Variations on genetic material cannot be studied morphologically, while RAPD technique may overcome such type of problems and is very effective for microbial species characterizations (Tiwari *et al.,* 2011). The results shown in dendogram are completely compatible with the geographical locations and belts from where the isolates have been collected. Lahore and Multan are distant but fall in the same belt in citrus producing areas of the Punjab. Lahore, Multan, Bhakar, Jhang and Sargodha fall in the same geographical belt, whereas Chiniot and Faisalabad render a separate region. Consequently the collected isolates from different regions form distinct groups showing a range of variability as seen by UPGMA results. Sahiwal being more close to southern Punjab showed more variation i.e., 23% compared to other isolates. Here another point of concern cannot be negated that the mutation frequencies fluctuate considerably along the genomic nucleotide sequences such that mutations focus at certain positions called hotspots. Mutation hotspots in DNA reflect the effect of the environmental factors in which the organism prevail (Rozin and Pavlov, 2003). These mutations are largely due to the point mutations, insertions, deletions, inversions and translocations in the chromosomes, which cause the variability in the genome that is easily detected through the fingerprinting process using various molecular marker tools such as RAPD.

The citrus producing belt is in extreme danger from *P. italicum* due to its most occurrences in storage conditions. Therefore, suitable integrated control measure should be taken to reduce this threat. Most probably the bio-control measures are more efficient, environmental safe and cost effective in a country like Pakistan. Accordingly, the future studies should be focused on the use of environmentally safer control measures.

References

Akintobi, A.O., I.O. Okonko, S.O. Agunbiade, O.R. Akano and O. Onianwa, 2011. Isolation and identification of fungi associated with the spoilage of some selected fruits in Ibadan, South Western Nigeria. *Acad. Arena*, 3: 1–10

Al-Hindi, P.R., A.R. Al-Najada and S.A. Mohamad, 2011. Isolation and identification of some fruit spoilage fungi: screening of plant cell wall degrading enzymes. *Afr. J. Microbiol. Res.*, 5: 443–448

Baiyewu, R.A., N.A. Amusa, O.A. Ayoola and O.O. Babalola, 2007. Survey of the post-harvest diseases and aflatoxin contamination of marketed pawpaw fruit (*Carica papaya*) in south western Nigeria. *Afr. J. Agric. Res.*, 2: 178–181

Bukar, A., M.D. Mukhtar and S. Adamu, 2009. Isolation and identification of postharvest spoilage fungi associated with sweet oranges traded in kano metropolis. *Bayero J. Pure Appl. Sci.*, 2: 122–124

Dillard, C.J. and J.B. German, 2000. Phytochemicals: nutraceuticals and human health. Review. *J. Sci. Agric*., 80: 1744–1756

Doyle, J.J., 1991. DNA protocols for plants. In: Molecular Techniques in Taxonomy, G. Hewitt, A.W.B. Johnson and J.P.W. Young (Eds.), pp: 283–293. NATO ASI Series H, Cell Biology 57. SpringerVerlag, Berlin, Germany.

Dupont, J., S. Magnin, A. Marti and M. Brousse, 1999. Molecular tools for identification of *Penicillium* starter cultures used in the food industry. *Int. J. Food Microbiol.*, 49: 109–118

Filtenborg, O., J.C. Frisvad and U. Thrane, 1996. Moulds in food spoliage. *Int. J. Food Microbiol.*, 33: 85–102

Giridher, P. and S.M. Ready, 1997. Incidences of mycotoxins producers on species from Andhra Pradesh. *J. Ind. Bot. Soc.*, 76: 161–164

Halmschlager, E., R. Messner, T. Kowalski and H. Prillinger, 1994. Differentiation of *Ophiostoma piceae* and *Ophiostoma quercus* by morphology and RAPD analysis. *Syst. Appl. Microbiol.*, 17: 554–562

Liu, Q., J. Xu, Y.Z. Liu, X.L. Zhao, X.X. Deng, L.L. Guo and J.Q. Gu, 2007. A novel bud mutation that confers abnormal patterns of lycopene accumulation in sweet orange fruit (*Citrus sinensis* L. Osbeck). *J. Exp. Bot.*, 58: 4161–4171

Messner, R., H. Prillinger, F. Altmann, K. Kopandic, K. Wimmer, O. Molnar and F. Weigang, 1994. Molecular characterization and application of random amplified polymorph DNA analysis of Markia and Sterigmatomyces species. *Int. J. Syst. Bacteriol.*, 44: 694–703

Montesinos-Herrero, C., M.A. Del Rio, C. Paster, O. Brunetti and L. Palou, 2009. Evaluation of brief potassium sorbate dips to control postharvest *Penicillium* decay on major citrus species and cultivars. *Postharvest Biol. Tec.*, 52: 117–125

Moss, M.O., 2008. Fungi, quality and safety issues in fresh fruits and vegetables. *J. Appl. Microbiol.*, 104: 1239–1243

Naseer, M., 2010. *Government of Pakistan, Report on Export of Kinnow*. Trade Development Authority of Pakistan, Faisalabad, Pakistan

Niji, D.V., H.P. Egmond, F.M. Rombouts and S.H.W. Notermans, 1997. Identification of Hazardous *Eusarium* secondery metabolites occurring in food raw materials. *J. Food Saf.*, 17: 161–191

Palou, L., A. Marcilla, C. Rojas-Argudo, M. Alonso, J.A. Jacas and M.A. Del Rio, 2007. Effects of X-ray irradiation and Sodium carbonate treatments on postharvest *Penicillium* decay and quality attributes of Clementine mandarins. *Postharvest Biol. Tec.*, 46: 256–261

Ranganath, H.R., G.P. Shyam and P. Sheeba, 2002. PCR-fingerprinting of some *Trichoderma* isolates from two Indian type culture collections – a need for re-identification of these economically important fungi. *Curr. Sci*., 83: 25

Rogozin, I.B. and Y.I. Pavlov, 2003. Theoretical analysis of mutation hotspots and their DNA sequence context specificity. *Mutat. Res.*, 544: 65–85

Sabir, H.M., M.B. Khan and Z Hussain, 2010. Marketing Margin of Mandarin: A Case Study of Sargodha Region, Pakistan. *Pak. J. Soc. Sci.*, 30: 275–291

Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. *Molecular Cloning: A Laboratory Manual*, 2nd edition. Cold Spring Harbor Laboratory, New York, USA

Samson, R.A. and J. Varga, 2007. *Aspergillus* systemics in the genomic era. *CBS Stud. Mycology,* 59 59: 206

Terol, J., A. Conesa and J.M. Colmenero, M. Cercos, F. Tadeo, J. Agusti, E. Alos, F. Andres, G. Soler, J. Brumos, D.J. Iglesias, S. Gotz, F. Leqaz, X. Argout, B. Courtois, P. Ollitrault, C. Dossat, P.Wincker, R. Morillon and M. Talon, 2007. Analysis of 13000 unique *Citrus* clusters associated with fruit quality, production and salinity tolerance. *BMC Genomics*, 8: 31

Tiwari, K.L., S.K. Jadhav and A. Kumar, 2011. Morphological and molecular study of different *Penicillium* species. *Middle-East J. Sci. Res.*, 7: 203–210

Tournas, V.H. and E Katsoudas, 2005. Mould and yeast flora in fresh berries, grapes and citrus fruits. *Int. J. Food Microbiol.*, 105: 11–17

Varga, J., B. Toth, K. Rigo, J. Teren, Z. Kozakiewicz and R.F. Hoekstra, 2000. Phylogenetic analysis of *Aspergillus* section Circumsdati based on sequences of the internal transcribed spacer regions and the 5.8 SrRNA gene. *Fungal Genet. Biol*., 30: 71–80

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