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Pathological and Molecular Characterization of Post Harvest Fungal Pathogens of Mango

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ABSTRACT

Present study focused on pathological and genetic characterization of predominant post harvest fungal pathogens causing stem end rot, anthracnose and side rots of mango. Aggressiveness of these fungal isolates was tested through artificial inoculations under controlled conditions and all isolates were proved pathogenic with varying degree of aggressiveness in reference to control on both Sindhri and White Chounsa variety with exception of Colletotrichum gloeosporioides which showed no disease symptoms and characterized as non-aggressive isolates. All pathological results were proved highly significant at P<0.05 through ANOVA. DNA of fungal pathogens was successfully extracted and amplification was done through ITS1 and ITS4 primers and the amplified amplicons were productively digested with restriction enzymes (MboI, AluI, EcoRI, HaeIII, TaqI). Good genetic variability was obtained among the isolates of Lasiodiplodia theobromae, Aspergillus niger and Aspergillus flavus but Colletotrichum gloeosporioides did not show genetic variability.

Introduction

Pakistani mangoes acquired a superior position in global race due to their good qualities, delicacy and the delicious varieties. According to statistical analysis, Pakistan is the fourth largest producer of mango fruit with exact value of 1,784,300 million tons (Minfal, 2009). Mango production is unfavorably hampered by the several biotic diseases in Pakistan. Among which post harvest diseases are the main risk to the mango cultivation. Post harvest losses occur due to infection by bacteria, fungi or certain physiological disorders (Wainwright and Burbage, 1989). Post harvest diseases of mango fruit reduce fruit quality and cause for severe economic losses as they lead to the entirely unmarketable fruits. Technologies used for post harvest processing, packaging, transportation, handling, storage and consumption in Pakistan are traditional which cause for 20-40% loss of fruits and vegetables. Regardless of the fact that conditions for mango production are favorable in Pakistan, predominantly in regions of Punjab and Sindh but diseases have rendered yield of mango crop significantly low (Shahbaz et al., 2009). Nearly 5% fruit is lost due to the post harvest diseases, while this figure can be 100% if the conditions are suitable for disease development (Johnson, 2008).

Main fungal pathogens that are involved in the mango rotting at post harvest stages include Colletotrichum species responsible for the mango Anthracnose; Botryodiplodia theobromae are causal agents of stem end rot. Stem end rot caused by Lasiodiplodia theobromae is a serious threat to the economy of a country (Lonsdale, 1993). Anthracnose caused by Colletotrichum species is regarded as one of the single most significant threat affecting a vast host range which includes fruits, vegetables and cereals and is considered as most dominant disease of the mango mainly in the humid production areas where the disease incidence may reach upto 100% (Ploetz and Freeman, 2009). Aspergillus niger, causal agent of Aspergillus rot have adverse effect on the fruit after harvesting.

During 2011 and 2012, mango diseased samples were collected from domestic markets and orchards of Punjab and Sindh and assessed for post harvest diseases incidence and associated fungal pathogens. Stem end rot, anthracnose and side rots are the predominant fungal diseases in Pakistan, requiring systematic study. Previously, considerable work has been done on the morphological identification and characterization of Colletotrichum, Aspergillus and Lasiodiplodia species at general level but on the genetic diversity through molecular technique has yet not been performed in Pakistan. A more detailed investigation was still called for understanding the relationship between aggressive behavior and genetic diversity of fungal pathogens involved in post harvest fungal diseases of mango.

Objectives of present study were (i) Analysis of pathogenic behavior of different fungal pathogens causing post harvest diseases of mango (ii) Genetic characterization of fungal pathogens through ITS-PCR-RFLP (iii) Relationship of pathogenic fungi on the basis of their molecular characterization.

Material and Methods

Fungal isolates

During 2011 and 2012, diseased mango fruit samples were collected from domestic markets and orchards of Punjab and Sindh, Pakistan. From the infected fruits associated fungal pathogens were isolated and identified as Aspergillus niger, Aspergillus flavus, Colletotrichum gloeosporioides and Lasiodiplodia theobromae. Samples were preserved in laboratory and utilized for pathological and molecular studies.

Pathogenicity tests

Pathogenicity tests were carried out on healthy mature green mango fruits in order to know the aggressive behavior of fungal pathogens. Two famous varieties, White Chounsa and Sindhri were selected for this purpose. Samples were surface sterilized by dipping in hot water at 52^{o}C for 5 minutes and left for some time to air dry. After that fruits were inoculated by using plug placement method for Lasiodiplodia theobromae, spore suspension method for Colletotrichum gloeosporioides and

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wounding method for Aspergillus species. In analysis mango fruits were inoculated with 1 week old culture and control samples were inoculated with sterilized agar plug. Experiment was conducted in complete randomized design (CRD) plan with three replicates and six treatments were used for each isolate. The inoculated fruits were incubated at 28°C in plastic bags with wet cotton plug. After 24 hours the inoculum was removed and incubated till fruit was covered with disease. The disease was observed for 3–10 days after inoculation (Lelliott and Stead, 1987). Resultant lesion width and length were combined in order to calculate the lesion area by using equation Area of oval = πlw (Corkidi et al, 2006). Reisolation of the fungi from inoculated mango fruit was done and compared with mother cultures in order to confirm the Koch's postulates (Lin et al., 2002).

Statistical Analysis

Data recorded for various characteristics were analyzed with CRD two factor factorial analysis of variance (ANOVA) technique using Statistix 8.1 software. For significant F value, LSD was used for mean comparison at 0.05 % level (Sakalidis et al., 2011).

Molecular Analysis

Isolation and Amplification of DNA

Total genomic DNA of 57 fungal isolates was isolated using modification of phenol extraction method. DNA concentration was estimated to 25ng through Lambda DNA standards (Reader and Broda, 1985). Gene 5.8S and two flanking ITS1 and ITS2 internal transcribed spacers were amplified by following the protocol proposed by Mohankumar et al. (2010) with some modifications. The primers used for the amplification were ITS1F (5'- TCC GTA GGT GAA CCT GCG G-3') (Gardes and Bruns, 1993) and ITS4R (5' TCC TCC GCT TAT TGA TAT GC-3') (White et al., 1990). The reaction mixture was made to a final volume of 50 µl which contained 25ng of template DNA, 20 pmol primers ITS1 & ITS4, 10mM of dNTPs, 5 µl PCR buffer with NH_4 $(SO_4)_2$, 5 µl $MgCl_2$ and 1U Taq DNA polymerase. PCR program followed for amplification was initial denaturation for 1 minute at 95°C, followed by 35 cycles of 1 minute each for denaturation at 95°C, annealing at 55°C and extension at 72°C while final extension of 7 minutes at 72°C. PCR products were analyzed electrophoretically and the amplified bands were compared with 1kb ladder. PCR products were purified with PCR purification kit (Fermentas).

RFLP analysis

RFLP analysis was carried out by following the modification of Maharaj et al. (2012) method. Restriction enzymes AluI, MboI and EcoRI for Lasiodiplodia, restriction enzymes AluI, EcoRI, HaeIII for Colletotrichum gloeosporioides and TaqI, HaeIII, EcoRI for Aspergillus flavus and Aspergillus niger were selected for determining the genetic variability among the isolates. For digestion RFLP reaction mixture was made which was consisted of 20µl of PCR reaction containing 10µl of ITS DNA template, 2µl of enzyme buffers, 2µl of restriction enzyme and 6µl of DEPC water. This reaction mixture was incubated at 37°C for 18 hours. Then the restriction fragments were separated on the 3.5% agarose gel and visualized under the UV light. Fragment sizes were evaluated against the standard 1kb ladder (Invitrogen).

Scoring and statistical analysis

Visible products of RFLP were scored from the image of the gels. Presence or the absence of bands was recorded. Similarity matrices were constructed after authentication of bands to compare the patterns. Minitab software was used to construct a dendrogram from the similarity matrices.

Results and Discussions

Pathogenicity of fungal isolates

The morphologically similar fungal isolates showed variable pathogenic responses towards the Sindhri and White Chounsa variety under controlled conditions. Lasiodiplodia theobromae and Aspergillus isolates were proved pathogenic through artificial inoculations methods on detached mango fruit with the exception of few Aspergillus isolates which showed no disease on White Chounsa. Apart from these none of Colletotrichum gloeosporioides isolates showed disease symptoms on any variety and characterized as non-virulent. On White Chounsa mangoes lesions appeared earlier than the Sindhri mangoes. The diseases were evaluated from the appearance of symptoms till half of the fruit (50%) was infected. Positive results for post harvest disease symptoms were obtained through artificial inoculations. Pathogenicity tests established the fact about disease development is supported by mechanical injuries, bruises or wounds as distinguished by Zitter, (1985). This finding is in line to the work of Kumar et al. (1993) who provided similar results on different fruits and plants and established the fact that wounding is required for disease. Also hail, birds, heavy rains and the insects increase the risks of injuries and also provides with opportunity for pathogen to penetrate in host system (Mahmood et al., 2007). Cell wall degrading enzymes are known to have a role in pathogenesis caused by bacteria as well as fungi on their respective hosts. Noteworthy evidences were reported regarding role of pectolytic enzymes (Have et al., 1998). The comparison of mean lesion produced by post harvest fungal pathogens showed that lesions were significantly larger than control at $P<0.05$ which leads to large standard error. But the lesions produced by isolates Lasiodiplodia and Aspergillus isolates were not significantly different among each other. And the fungus that was inoculated, successfully re-isolated from infected fruit supporting Koch's postulates. The lesions produced by L. theobromae were brownish black and started from stem (collar) region and spread linearly along the fruit resulting in the softening of skin and pulp became watery which could be punctured with finger. Lesion size observed was quite variable 5.3-188.6 cm^2 (White Chounsa) and 6.9–68.5 cm^2 (Sindhri).The side rots symptoms caused by A. niger and A. flavus started from the area of inoculation and spread linearly with appearance of blackish brown blemishes. Sometimes fruit skin may become wrinkled and sunken with typical rot smell. Lesion sizes were variable 8.0 - 64.5 cm^2 (White Chounsa) and 0.3–77.3 cm^2 (Sindhri). Present study revealed the extensive association of fungal isolates with mango losses after harvesting in Pakistan. Reliable correlation of the host pathogen with origin was not established due to variation in aggressive behavior of isolates prevailed in every case. The reason behind considering Sindhri and White Chounsa for pathogenicity trails was that these two varieties of Pakistan is regarded as the exporter commodity and in regard to the demand of exporters who want comprehensive data on shelf life of mango and their resistance towards rots.

Results showed that Sindhri variety is more resistant to rots and this is in line with previous studies from all over the world (Malik et al., 2005). This resistance to rots could be due the fact that Sindhri variety is hard in texture while White Chounsa variety has soft texture and prone to rots.

Pathogenicity of Lasiodiplodia theobromae

The pathogenicity trial on mango fruit proved Lasiodiplodia isolates highly aggressive towards White Chounsa. Largest lesion area was showed by the isolates of Rahim Yar Khan). Least aggressiveness was showed by the isolates of Shaheed

Benazirabad and Sanghar but still larger than control. These three isolates belonged to Sindh (Shaheed Benazirabad and Sanghar).

On the basis of aggressive behavior of Lasiodiplodia isolates on Sindhri, largest lesion area was showed by the isolates belonging to Punjab (Rawalpindi and Rahim Yar Khan). Least aggressiveness was showed by the isolates of Sindh (Sanghar) (fig. 1) . The variation in infection depends on physiological maturity of fruit. Pathological and morphological characterization of Lasiodiplodia theobromae, Aspergillus niger and Colletotrichum gloeosporioides revealed these isolates were similar but not identical in morphological characters but showed high pathogenic variability. The variability in pathogenic behavior of isolates of same species is due to evolution among isolates. Similar results were presented by Shah et al., (2010). He studied thirteen isolates of Lasiodiplodia theobromae in terms of their morphological and the pathological characterization isolated from the pear fruit grown in the Punjab. The pathogenicity trails were carried out on fruit. Mean lesion length of different isolates was ranged from 1.9-7.2x0.8-3.3 cm. Pathogenicity results demonstrated that isolates of L. theobromae were the most pathogenic towards mango. This is in clear agreement with pathogenic studies conducted on L. theobromae isolates of mango by Sakalidis et al. (2011).

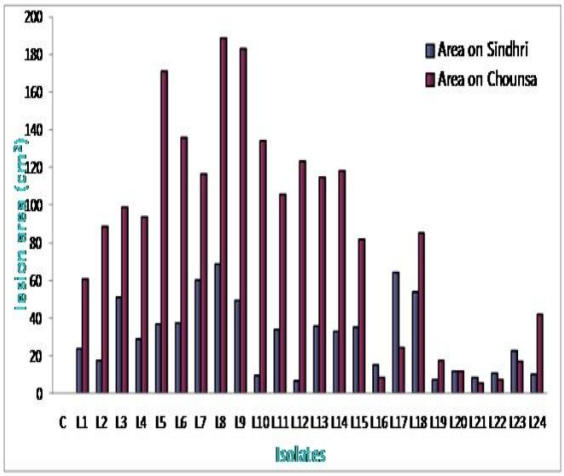

Figure 1. Mean aggressiveness comparison of L. theobromae isolates causing post harvest fungal diseases on White chounsa and Sindhri

Pathogenicity of Colletotrichum gleoesporioides

No infection showed by Colletotrichum gloeosporioides isolates and it could be due to non-pathogenic behavior or unfavorable environmental conditions provided. Similarly, Timmer et al. (1998) also found C. acutatum isolates did not produce anthracnose disease on citrus fruit that were isolated from citrus.

Pathogenicity of Aspergillus niger and Aspergillus flavus

On White chounsa, isolates of Aspergillus showed different disease symptoms Isolates from Punjab (Rahim Yar Khan and Shujabad) showed largest lesions. While isolates from Sindh (Sanghar) showed the smallest lesions.

Different isolates of Aspergillus showed different aggressiveness on Sindhri mangoes. Isolate AN19 showed largest lesion area (cm^2), 77.318 cm^2 which belonged to Punjab (Shujabad). AN12, AN13, AN21 and AN22 showed no disease symptoms and among these AN12 belonged to Sindh (Sanghar) while remaining three belonged to Punjab, (Rahim Yar khan, Multan and Rawalpindi). Isolate AN15 showed least disease 0.3908cm^2 and it belonged to Sindh (Sanghar) (Fig. 2). Aspergillus isolates obtained from Punjab were proved more aggressive and produced larger lesion on mango fruit. They produced quick disease symptoms which are supported by the study of Akintobi et al. (2011). He observed that Aspergillus niger appeared first on fruits before any other fungi.

Isolates from Punjab were proved more aggressive towards the fruit than the isolates from Sindh and it is due to the fact that in Punjab orchards are relatively smaller and closer to each other while in Sindh the situation is reciprocal (Meer et al., 2013). Another important reason for more aggressive behavior of Punjab isolates is the excessive use of nitrogen fertilizers. Conditions in Punjab are hot and dry which favors mango growth while m in Sindh where conditions are hot and humid with minor averages in frost free pockets of Baluchistan and KPK.

The environmental conditions plays very crucial role in disease development and pathogens spread as weather is hot and humid in Pakistan because environmental factors, harvesting techniques, packaging, transportation and marketing all factors are in the favor of pathogens attack and destroy the fruit quality.

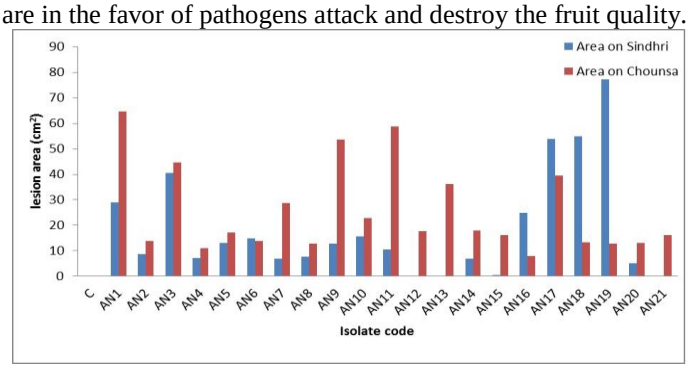

Figure 2. Mean aggressiveness comparison of Aspergillus species isolates causing post harvest fungal diseases on White chounsa and Sindhri

Molecular characterization of Post-Harvest Fungal Pathogens

DNA Extraction and Amplification

Sufficient amount of the pure fungal DNA having high molecular weight was obtained by this process. Isolated DNA was diluted accordingly to 25ng. The amplified products (which comprised of single amplification band) for L. theobromae, C. gloeosporioides and Aspergillus species were in the range of 580-600bp, 600 bp and 600-650 bp respectively. Successful amplification of rDNA was achieved with ITS1 and ITS4 primers. This is in agreement with previous study (Ismail et al., 2012). The rDNA have multi-copy genes within it and it undergoes rigorous independent evolution as a result of which sequence similarity and the homogeneity exists within species. These sequence regions evolved at different rates that helped in taxonomic identifications (Prusky, 2000). Coding regions within the fungal rDNA genes 18S, 5.8S, and 28S are quite conserved and evolve slowly in different fungi. While non-coding regions i-e internally transcribed spacer (ITS1 and ITS2) evolve rapidly so here rates of evolution vary between species of different genera as well as among species of same genus (Roth, 1998). ITS rDNA region is widely used for determining genetic variability within the fungi at specie and sub-specie level (Karthikeyan et al., 2009).

Restriction Enzyme Digestion of ITS Amplified Amplicons

Restriction Enzyme Digestion of Amplified Amplicons of Lasiodiplodia theobromae. Amplified fragments of L. theobromae isolates were digested by restriction enzymes MboI, AluI and EcoRI. These restriction enzymes provide considerable genetic diversity among fungal genomes. Restriction enzyme MboI and AluI showed total polymorphism while EcoRI enzyme

also showed monomorphism with some isolates. RFLP analysis of rDNA ITS regions has been utilized for studying genetic diversity within isolates of the fungi (Appiah et al., 2004).

Successful extraction of pure genomic DNA of fungi is important for the PCR, genetic diversity. The obtained quality, concentration of the DNA was sufficient for obtaining ITS-PCR products as well as distinct DNA patterns through RFLP analysis. At species level the genetic diversity is a prerequisite for survival and development. Studying the genetic diversity and genetic structure of a species is the basis of exploring its adaptability and viability (Shen et al., 2005).

MboI: Three types of diversity patterns were produced by MboI and exhibited seven restriction sites in their genome (fig. 3a).

EcoRI: Least variations were achieved by EcoRI and produced only 2 distinct types of diversity patterns and up to two restriction sites for each isolate (fig. 3b).

AluI: AluI also provides three types of restriction patterns which were achieved based on genetic diversity and contained upto four restriction sites for each isolate (fig. 3c). The grouping of above isolates was diverse irrespective of their geographic origin and genetic makeup. The grouping was made irrespective of their origin and this is analogous to studies on Fusarium isolates that showed no correlation among isolates and the host cultivars (Iqbal, 2004). This clustering in one group means they share high level of similarity in their genomes and behavior. Different restriction patterns obtained showed the possible genotypes among fungal isolates. Results depicted that fungal isolates were not host and area specific instead these are distributed throughout the country by means of air borne conidia dispersal from their origin to other places (Mahmood et al., 2007). Isolates showed disease on both varieties it means they have broad spectrum and showed diversity in genomes and not host specific. Like isolate L2, L3 and L20 were in same cluster among which isolate L2, L3 were from Punjab (Shujabad and Multan) while isolate L20 was from Sindh. Isolate L13 from Faisalabad and L19 from Sanghar shared one cluster representing two different provinces. Only Isolate L12 and L14 (Punjab) shared same genetics and aggressive behavior towards White Chounsa Mango fruit. And isolate L5 and L6 (Punjab) shared same genetics and aggressive behavior towards Sindhri mango (fig. 3d). Good genetic variability among the L. theobromae isolates was reported by Al-Adawi et al. (2003).

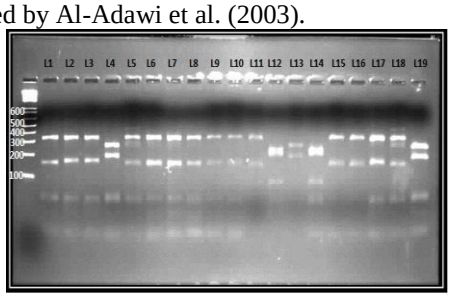

Figure 3a. Lasiodiplodia theobromea isolates digested amplicons pattern with MboI enzyme

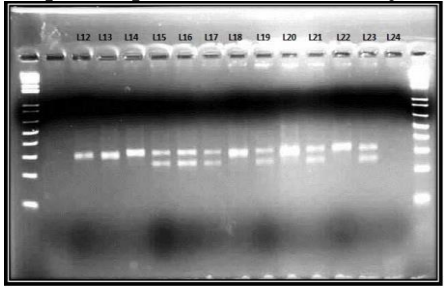

Figure 3b. Lasiodiplodia theobromea isolates digested amplicons pattern with EcoRI enzyme

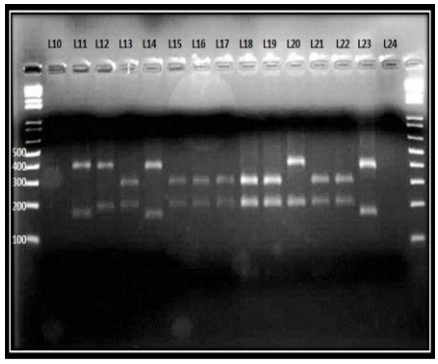

Figure 3c. Lasiodiplodia theobromea isolates digested amplicons pattern with AluI enzyme

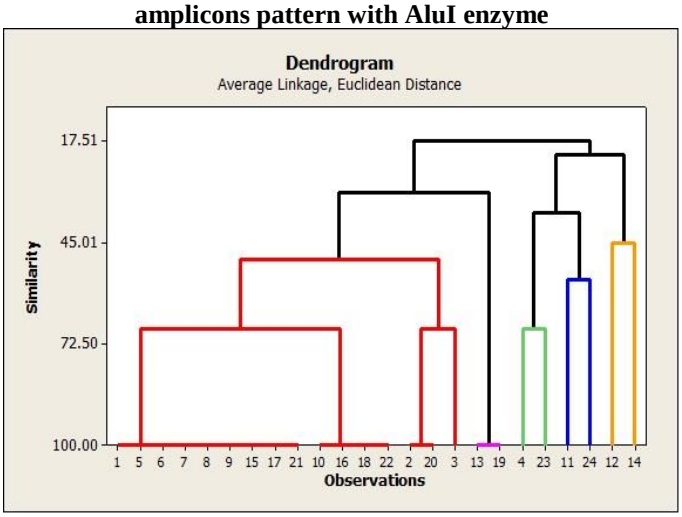

Figure 3d. Phylogenetic Dendrogram constructed from RFLP restriction enzyme (AluI, EcoRI and MboI) data indicating relationship among post-harvest fungal isolates of Lasiodiplodia theobromae

Restriction Digestion of Amplified Amplicons of Colletotrichum gloeosporioides

Similar to pathogenic behavior, isolates of Colletotrichum gloeosporioides did not show genetic diversity after the digestion of amplified fragments by digestion with restriction enzymes (HaeIII, AluI and EcoRI). All isolates of Colletotrichum showed similar restriction pattern (fig. 4abc). In case of Colletotrichum gloeosporioides no variation was showed by any of the restriction enzyme and this can be due to the uniform asexual population. This is in agreement with previous studies of C. acutatum and C. fragariae isolates from the strawberries in which very little variation in genomic DNA was observed in each species by consuming different molecular techniques (Freeman and Rodriguez, 1995).

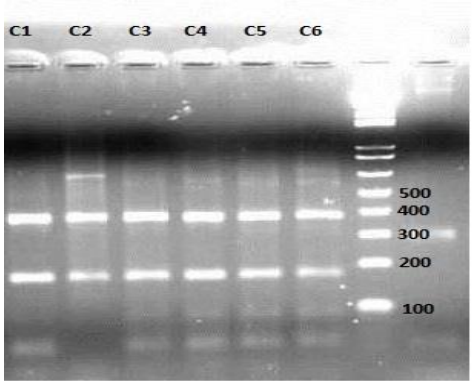

Figure 4a. Colletotrichum gloeosporioides isolates digested amplicons pattern with HaeIII enzyme

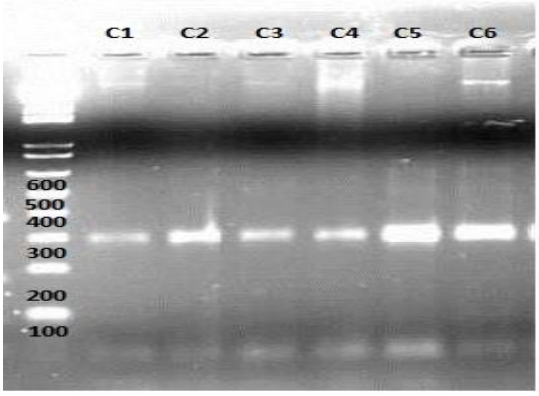

Figure 4b. Colletotrichum gloeosporioides isolates digested amplicons pattern with EcoRI enzyme

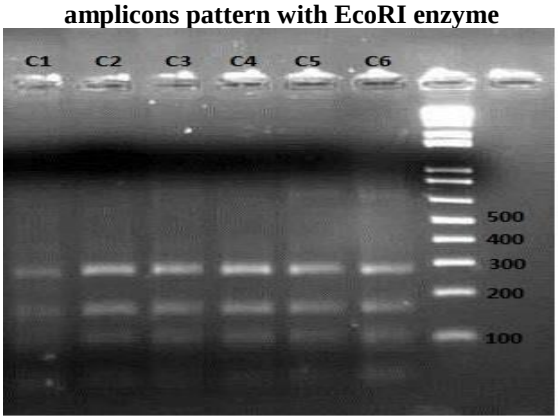

Figure 4c. Colletotrichum gloeosporioides isolates digested amplicons pattern with AluI enzyme

Restriction Digestion of Amplified Amplicons of Aspergillus niger and Aspergillus flavus

Restriction enzymes HaeIII, TaqI and EcoRI yielded a high degree of genetic variability among the isolates of Aspergillus. A variation in the banding pattern was reflected by these isolates conflicting to cultural and morphological similarities. The diversity among the isolates can be linked to non-specific or wider host range. Genetic diversity among fungal population is very important in devising suitable management strategies because different subgroups and species vary in sensitivity to the fungicides (Van Hemelrijck et al., 2010) thus facilitating producers in reducing economic losses. This knowledge will also helpful for the breeding programs regarding genetic resistance to these post harvest diseases in mango, considering that obtaining stem end , anthracnose and side rot resistant mango will be the best control outcome.

HaeIII: Restriction enzyme HaeIII produced highest degree of diversity containing upto eight restriction sites and produced eight diverse restriction patterns (fig. 5a).

TaqI: Genetic variability was achieved by TaqI and yielded diversity containing up to four restriction sites and produced four restriction patterns (fig. 5b).

EcoRI: By the digestion of amplified product by EcoRI, restriction pattern is quiet similar among the isolates of Aspergillus and yielded two restriction patterns and exhibits same number of restriction sites (fig. 5c). For some isolates monomorphism was produced same like with some isolates of Lasiodiplodia theobromae. EcoRI showed monomorphism with some of the isolates this can be due to the possibility that it produced the restriction pattern of equal size which when moved on gel could be mixed and appeared as single band this observation is in line with Maharaj et al. (2012). Isolates joined clusters irrespective of their geographic origin. The similarity found between isolates of Aspergillus ranged from 21-100%. Isolate AN1, AN26, AN27, AN28 joined cluster 1 and they were from Punjab and Sindh. Isolate AN1 belonged to Shujabad, Punjab while remaining three belonged to Sanghar, Sindh. Isolate AN6, AN23, AN24 shared 100% similarity and fall in cluster with AN7 isolate among which AN6, AN7 belonged to Rahim Yar khan while AN23 and AN24 from Sanghar and Shaheed Benazirabad, Sindh. Isolates AN14 and AN17 fall in one cluster and they were also representing Punjab and Sindh. Isolates AN2, AN3, AN9, AN11, AN12, AN15, AN22, and AN25 shared 100% similarity and made one larger cluster and comprised of isolates from both provinces as well as of same location (figure 19). So grouping was irrespective of the origin. Isolates having same aggressive behavior also fall in different clusters on grouping according to their genetic diversity pattern. Like isolate AN1, AN16, AN27 shared one cluster according to their aggressive behavior but with genetic diversity isolate AN1 and AN27 shared similar group while AN16 joined separate group. Some relationship was obtained between the aggressive behavior of isolates and their genetics. There are few isolates that shared 100% similarity in their aggressiveness and genetic makeup. Isolate AN1and AN27 (Punjab and Sindh) shared same cluster in both genetic analysis and aggressive behavior on Sindhri Mango. Isolate AN9 and AN11 shared same cluster with 100% similarity in genetic analysis and aggressive behavior on both mango varieties Sindhri and White Chounsa. Isolate AN15, AN22 and AN6, AN24 shared same genetic analysis and aggressive behavior on Sindhri Mango. While Isolate AN8 and AN19 shared genetics and aggressive behavior on White Chounsa. Aspergillus species showed considerable variation (fig. 5d). These results of genetic variability and aggressiveness assays confirmed that distinct groups are present in the Lasiodiplodia theobromae and Aspergillus species. Some similarity relationship exists within the aggressiveness and the genetic variability. Apparently no considerable correlation was found with geographic origin and the aggressiveness. These results were in line with the study of (Carter et al., 2002) on Fusarium graminearum and with Namiki et al. (1998). Molecular and pathological studies are serving to resolve the relationships among the diversity of Lasiodiplodia theobromae, Colletotrichum gloeosporioides and Aspergillus species but studies to date are only preliminary. Numerous questions remain concerning evolutionary relationships among the genus (Cunnington et al., 2004). Their cross-infection ability as these fungi has diverse range of hosts. Further molecular investigations consuming other genes, more precise and sensitive approaches are still required. Here effort was made with incorporation of three enzyme set for genetic variability but still required large set of enzymes for diversity pattern and knowledge of total restriction sites will be obtained

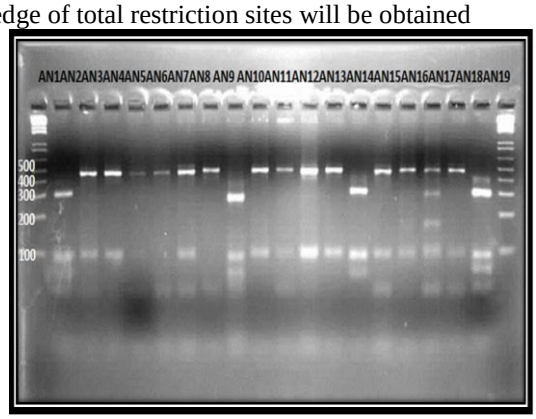

Figure 5a. A. niger and A. flavus isolates digested amplicons pattern with HaeIII enzyme

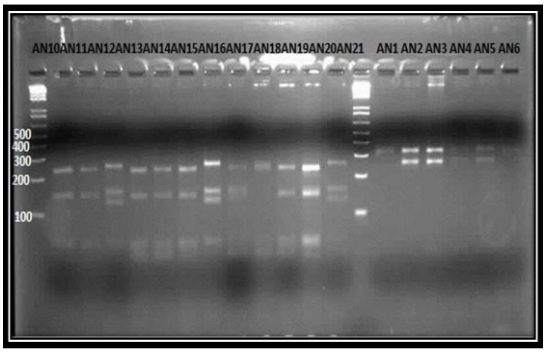

Figure 5b. A. niger and A. flavus isolates digested amplicons pattern with with TaqI and EcoRI enzyme

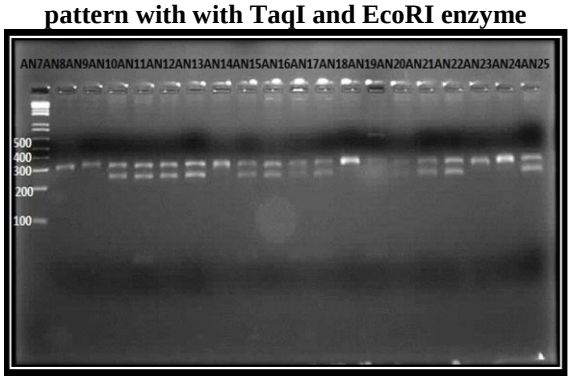

Figure 5c. A. niger and A. flavus isolates digested amplicons pattern with EcoRI enzyme

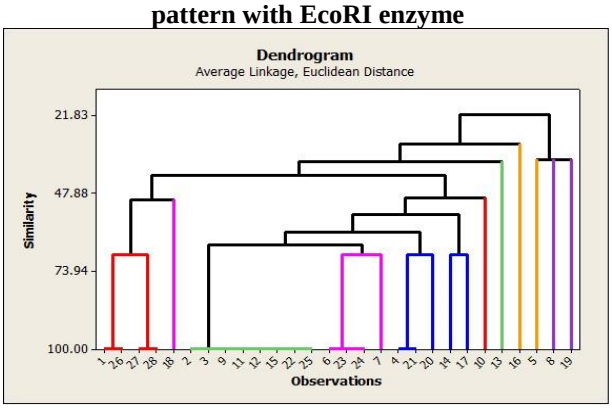

Fig 5d. Phylogenetic Dendrogram constructed from RFLP restriction enzyme (TaqI, EcoRI and HaeIII) data indicating relationship among post harvest fungal isolates of Aspergillus species

Conclusion

By pathological analysis it was proved that wounding is important for disease development and environmental factors played very crucial role. In genetic characterization, RFLP has a good potential for determining genetic variability among the post harvest fungal pathogens and different fungal clones can be differentiated by this technique. In present research very small relationship was found among the pathological and genetic characterization of post harvest fungal pathogens of mango. More surveys and detailed investigations about the pathogenic behavior and genetic variability of fungal pathogens are required for the development of management policies.

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