Physiological and Molecular Plant Pathology

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DOI:http://dx.doi.org/10.1016/j.pmpp.2013.06.001

Evaluation of genetic diversity in *Magnaporthe grisea* populations adapted to finger millet using Simple Sequence Repeats (SSRs) markers

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ABSTRACT

Finger millet blast caused by *Magnaporthe grisea*(anamorph: *Pyricularia grisea*) is a great threat to finger millet production worldwide. Genetic diversity and population structure of 72*M. grisea*isolates collected from finger millet (56), foxtail millet (6), pearl millet (7) and rice (3) frommajor crop growing areas inIndiawas studied using 24 SSR markers. None of the SSRs detected polymorphism in the *M. grisea* isolates from pearl millet. Seventeen SSR markers were polymorphicin the 65 non pearl millet isolates anddetected 105 alleles, of which one was rare, 83 common, 9 frequent and 12 most frequent. A model-based population structure analysis of the genomic data identified two distinct populations with varying levels of ancestral admixtures among the 65*M. grisea*isolates. Analysis of molecular variance (AMOVA)indicated that 52% of the total variation among the isolates used in this study was due to differences between the pathogen populations adapted to different hosts, 42% was due to differences in the isolates from the same host, and the remaining 6% due to heterozygosity within isolates. High genetic variability present in *M. grisea* isolates calls for the continuous monitoring of *M. grisea* populations anticipating blast resistance breakdown in finger millet cultivars grown in India.

Key words:Genetic diversity, Simple sequence repeats, *Magnaporthe grisea*, *Eleusine coracana*

Highlights:

- Seventeen of the 24 SSR markers were polymorphic and detected 105 alleles in the 65 *Magnaporthegrisea*isolates.
- Cluster analysis of SSR data classified the isolates into three major groups that corresponded with the host specificity.
- A model-based population structure analysis identified two distinct populations with varying levels of ancestral admixtures.

1.0. Introduction

Finger millet (*Eleusine coracana* L. Gaertn) is a widely grown grain cereal in the semi-arid areas of East and southern Africa and South Asia under varied agro-climatic conditions [1]. Finger millet is being increasingly recognized as apromising source of micronutrients and protein [2] forweak and immune-compromised people [3]. Besides energy, it contributes to alleviating micronutrient and protein malnutrition also called 'hidden hunger' affecting half of the world's population, especially women and pre-school children in most countries of Africa and South-east Asia [4]. Malnutrition due to protein deficiency is alsofound at alarming rates in the Indian subcontinent [5]. Although finger millet is tolerant to many biotic and abiotic stresses, the crop is severely affected by blast disease caused by an ascomycete fungus *Magnaporthe grisea* (Hebert) Barr. (anamorph: *Pyricularia grisea* (Cooke) Sacc.), which is very prominent among the constraints that affect yield, utilization and trade of finger millet within East Africa and South Asia [6,7]. Many of the widely grown landraces and high yielding varieties are susceptible to blast with yield losses of 10-50% being common [3] and losses canbe as high as 80-90% in the endemic areas [8]. The disease affects the crop at all growth stages from seedling to grain formation, withpanicle blast being the most destructive form of the disease [9,10]. *M. grisea* is pathogenic to more than 50 graminaceous hosts including food security crops such as rice, wheat, finger millet, pearl millet and foxtail millet [11,12]. Despitethewide host range of the pathogen, *M. grisea* populations mainly exist as host-specific (adapted) forms, capable of infecting a single host [13,14]. While some researchers have demonstrated successful infection of a host by anisolatefrom a different host under experimental conditions [15,16], others failed to confirm the results [13].

In thecase of finger millet, blast management through host resistance is very economical and relevant for the resource-poor and marginal farmers who cannot afford other methods of disease control such as use of expensive chemical fungicides. However,

resistance breakdown is a greatchallenge while breeding for blast resistance in finger millet because of pathogenic variation in *M. grisea*. It is important not only to develop cultivars with durable resistance, but also to monitor virulence change in the pathogen populations to anticipate resistance breakdown in existing finger millet cultivars, and to designstrategies to sustain cultivation of high yielding,farmer and consumer preferred cultivars [17]. Lack of knowledge on the pathogen adapted to finger millet in India has hindered efforts towards identification and development of resistant cultivars adapted to local agro-ecological conditions. Consequently, research efforts have focused on understanding the *M. grisea* population structure by combining modern molecular-biotechnological approaches with traditional pathological assays. Substantial work has been done in the rice-blast pathosystem, whereas such studies are very limited for the finger millet-blast pathosystem[3,7,14]. In order to measure genetic variability more precisely, molecular markers thatprovide an unbiased estimate of total genomic variation and have the potential to minimize errors due to sampling variance have been developed [18]. Furthermore, determination of fungal genetic diversity based on molecular markers is reliable as it is independent of culture conditions. DNA fingerprinting techniques have created new tools for the molecular analysis of *M. oryzae* populations [19] and this is equally applicable to *M. grisea* populations adapted to finger millet.

Assessment of genetic diversity in *M. grisea* from different crops has mostly relied on use of clones of the transposon MGR as a probe to detectrestriction fragment length polymorphism (RFLP), which is an expensive and time-consuming approach. Simple sequence repeats (SSRs) or microsatellites are PCR-based molecular markers, which may be more desirable for population genetic analysis because this approach makes it simpler to obtain accurate polymorphic data due to co dominance. Besides, these markers are highly reproducible, locus-specific, multi-allelic and abundant in animal, plant and

microbialgenomes [20]. Although generation of SSR markers is a time-consuming, labor-intensive and expensive task, several SSR markers have already been developed for *M. grisea* infecting rice [21–24]. However, SSRs have not been used to investigate pathogen populations adapted to finger millet. Prior few studies have examinedgenetic diversity in finger millet-infecting populations of *M. grisea*using MGR-RFLP [14], AFLP [3] and RAPD markers [7]. Here, we analyzedfinger millet infecting populations of *M. grisea*, collected from Andhra Pradesh, Bihar and Karnataka, India along with *M. grisea* isolates from pearl millet, foxtail millet and rice using SSR markers to (i) assess extent of genetic diversity in finger millet-infecting populations of *M. grisea* (ii) investigate genetic relatedness among*M. grisea* populations adapted to finger millet, foxtail millet, pearl millet and rice.

2.0. Material and Methods

2.1. Pathogen isolates

Blast infected (leaf, neck and finger) samples of finger millet, foxtail millet and rice were collected from Vizianagaram, Patancheru, and Nandyal in Andhra Pradesh, Mandya and Naganahalli in Karnataka, and Dholi in Bihar, India during 2008-10 rainy seasons (Table 1). In addition, seven *M. grisea* isolates from four major pearl millet growing states in India – Rajasthan, Haryana, Maharashtra and Uttar Pradesh [25] were also included in this study (Table 1). Isolations of *M. grisea* were made from the blast-infected tissue on oatmeal agar medium (rolled oats 50 g, agar 15 g, distilled water 1 L) and incubated at 25±1°C for 15 days. After incubation, a dilute spore suspension (3×10^3 spores/ml) was prepared in sterile double-distilled water and plated onto 4% water agar in Petri plates. Single germinating conidia were marked after 10-12 h of incubation under a microscope and transferred to test tubes containing oatmeal agar for further studies.

2.2. Isolation of genomic DNA

Isolates of *M. grisea* were grown in 2X yeast extract glucose (YEG) medium [14] in shake culture for 7-10 days at 25°C. Mycelia were harvested by filtration through Whatman filter paper No. 1, dried on blotting papers and ground to a fine powder in liquid nitrogen with a pre-cooled pestle and mortar. Genomic DNA was extracted from 200 mg of powdered mycelium of each isolate using CTAB (cetyltrimethylammonium bromide) method as suggested by Viji et al. [14]. The quantity and quality of the extracted DNA was assessed by running the DNA on 1% agarose gel, stained with ethidium bromide and photographed under UV illumination.

2.3. SSR genotyping

Twenty-four SSR markers (*Pyrms7-8*, *Pyrms 15-16*, *Pyrms33-34*, *Pyrms37-38*, *Pyrms 39-40*, *Pyrms41-42*, *Pyrms 43-44*, *Pyrms 45-46*, *Pyrms47-48*, *Pyrms 59-60*, *Pyrms 61-62*, *Pyrms 63-64, Pyrms 67-68, Pyrms 77-78, Pyrms 81-82, Pyrms 83-84, Pyrms 87-88, Pyrms 93-94, Pyrms 99-100, Pyrms 101-102, Pyrms 107-108, Pyrms 109-110, Pyrms 115-116* and *Pyrms125-126*) [22] were used for analyzing the SSR diversity in *M. grisea*isolates (Table 2). The forwardprimers were synthesized by adding M13-forward primer sequence (5'CACGACGTTGTAAAACGAC3') at the 5'end of each primer. PCR was performed in 5 µl reaction volume with final concentrations of 5 ng of DNA, 2.5 mM $MgCl_2$, 0.2 mM of dNTPs, 1X PCR buffer, 0.006 pM of M13-tailed forward primer, 0.09 pM of M13-Forward primer labeled with either 6-Fam or Vic or Ned or Pet (Applied Biosystems), 0.09 pM of reverse primers and 0.1 U of *Taq* DNA polymerase (SibEnzyme Ltd., Russia) in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, USA) with the following cyclic conditions: initial denaturation at 94˚C for 3 min then 10 cycles of denaturation at 94˚C for 1 min, annealing at 61˚C for 1 min (temperature reduced by 1˚C for each cycle) and extension at 72˚C for 1 min. This was followed by 40 cycles of denaturation at 94˚C for 1

min, annealing at 54˚C for 1 min and extension at 72˚C for 1 min with the final extension of 10 min at 72˚C. The PCR products were tested for amplification on 1.2% agarose.

Based on their expected amplicon size and/or dye, PCR products were pooled together along with internal size standard (GeneScan™ 500 LIZ® from Applied Biosystems) and capillary electrophoresis was carried out using ABI 3730*xl* Genetic Analyzer (Applied Biosystems, USA). Raw data produced from theABI 3730*xl* Genetic Analyser was analysed using Genemapper software (Applied Biosystems, USA) and fragment size was scored in base pairs (bp) based on the relative migration of the internal size standard.

2.4. Determination of allele frequency and diversity analysis

The alleles for each SSR locus across the samples were scored in terms of fragment length of the PCR amplified product in base pairs and used to calculate the basic statistics such as polymorphic information content (PIC), allelic richness as determined by a total number of the detected alleles, major allele frequency (M_{AF}), number of alleles per locus, gene diversity (GD), heterozygosity (H) and occurrence of unique, rare, common, frequent and most frequent alleles using PowerMarker version 3.25 [26].These estimates were performed across all the *M. grisea* isolates, and separately among isolates from different hosts. Unique alleles are those that are present in one isolate or one group of isolates but absent in other isolates or group of isolates. Rare alleles are those whose frequency is ≤ 1% in the investigated isolates. Common alleles have>1%-20% frequency while those occurring with >20 -50% and >50% frequencies were classified as frequent alleles and most frequent alleles, respectively.

2.5. Unweighted Neighbor-joining tree

The allelic data were converted into a binary matrix using the scores 1/0 for presence/ absence of the allele. A similarity matrix was generated from the binary data using Jaccard

similarity coefficient in the SIMQUAL program to cluster the isolates usingNTSYS-pc package [27].

2.5. Analysis of molecular variance (AMOVA)

Analysis of molecular variance for the*M. grisea* isolates from different hosts collected from different locations was performed using the software ARLEQUIN [28].

2.6. Population structure analysis

A set of 17 SSR markers were used to dissect the population structure of*M. grisea* isolates from finger millet, foxtail millet and rice. In order to infer the population structure of the*M. grisea* isolates without considering the host origin, the analysis was performed using the software package STRUCTURE version 2.3.4(http://pritch.bsd. uchicago.edu/structure.html) [29]. This method uses multilocus genotypes to infer the fraction of an isolate's genetic ancestry that belongs to a population for a given number of populations (*K*). The program STRUCTURE implements a model based clustering method for inferring population structure using isolate data consisting of unlinked markers to identify *k* clusters to which the program then assigns each individual isolate. To determine most appropriate *K* value, burn-in Markov Chain Monte Carlo (MCMC) replication was set to 300,000 and data were collected over 200,000 MCMC replications in each run. Three independent runs were performed setting the number of population (*K*) from 2 to 15 using a model allowing for admixture and correlated allele frequencies. The basis of this kind of clustering method is the allocation of individual genotypes to *K* clusters in such a way that linkage equilibrium isvalid within clusters, whereas this kind of equilibrium is absent between clusters. The *K* value was determined by LnP(D) in STRUCTURE output based on the rate of change in LnP(D) between successive *K*. The model choice criterion to detect the most probable value of *K* was Δ*K*, which is an ad hoc quantity related to the second-order change in the log probability of data (Ln P(D)) with

respect to the number of clusters inferred by Structure [30].The MCMC chain was run multiple times, using a correlated allele frequency model (prior mean = 0.01, prior SD = 0.05 and Lambda = 1.0) in the advance option of the STRUCTURE version 2.3.4.

3.0. Results

3.1. Polymorphic SSRs among M. grisea isolates

For assaying allelic diversity in 72 *M. grisea* isolates, a total of 24 SSR markers were used. However, only 17 (74%) produced clear, scorable and polymorphic markers among *M. grisea* isolates from different hosts and locations (3 pairs amplifieda product in all 72 isolates). The remaining seven (26%) primer pairs (*Pyrms 33-34, Pyrms 39-40, Pyrms 43-44, Pyrms 81-82, Pyrms 83-84, Pyrms 101-102* and *Pyrms 115-116*) were found monomorphic in all*M. grisea* isolates. None of the primer pairs detected polymorphism in pearl millet infecting *M. grisea*populations, butonly three SSR markers (*Pyrms 47-48, Pyrms 63-64* and *Pyrms 67-68*) amplified DNA frompearl millet isolates.Thus, isolates from pearl millet were excluded from further study. One SSR marker (*Pyrms 43-44*) amplified onlyfoxtail millet isolates. A high level of polymorphism was observed for 17 SSRs in the 65 isolates of *M. grisea* from finger millet, foxtail millet and rice (Table 1); thus, these SSRs and isolates were selected for further studies (Table 2).

3.2. Allelic richness and diversity in M. grisea

The 17 polymorphic SSR markers detected total 105 alleles in the 65 *M. grisea* isolates assayed. The number of alleles per locus ranged from 2 (*Pyrms 37-38*) to 13 (*Pyrms 15-16*) with an average of 6.18 alleles/locus (Table 2). The allele size ranged from 119 to 384 bp. The polymorphic information content (PIC) values varied from 0.205 (*Pyrms 37-38*) to 0.805 (*Pyrms 67-68*) with an average of 0.486/marker. Three markers *Pyrms 15-16*, *Pyrms 61-62* and *Pyrms 67-68* were highly polymorphic. Gene diversity, defined as the probability

that two randomly chosen alleles from the population are different, varied from 0.232 (*Pyrms 37-38*) to 0.827 (*Pyrms 67-68*), with an average of 0.517. A very low level of heterozygosity (0.000 to 0.046) was detected in *M. grisea* isolates but for *Pyrms 45-46* which detected 0.586 heterozygosity. Seven SSR loci detected no heterozygosity while nine detected <0.05 heterozygosity.

Of the 105 alleles detected in *M. grisea* isolates, only one was rare, 83 common, 9 frequent and 12 were most frequent. Common alleles were detected at all 17 SSR lociranging from 1 (*Pyrms 37-38*) to 12 (*Pyrms15-16*) with an average of 4.88 alleles per locus while frequent alleles ranged from 1 to 2 with an average of 0.52frequent alleles per locus. Most frequent alleles were detected atall the SSR loci except *Pyrms 15-16, Pyrms 47-48, Pyrms 59-60, Pyrms 61-62* and *Pyrms 67-68* with an average of 0.70 alleles per locus (Table 2).

3.3. Diversity in M. grisea populations adapted to different hosts

Of the 105 alleles detected in the 65 *M. grisea* isolates, 75 (one rare, 51 common, 10 frequent and 13most frequent)were from fifty-six fingermillet isolates, 44 (22 common, 12 frequent and 10 most frequent alleles) from six foxtail millet isolates and 15 most frequent alleles from three rice isolates (Table 3). The number of alleles per locus in finger millet isolates ranged from 2 to 13 with an average of 4.41 alleles; whereas in foxtail milletisolates, it ranged from 1 to 4 with an average of 2.75.The PIC value ranged from 0.067 to 0.759 (average 0.369) in finger millet isolates, 0.0 to 0.620 (average 0.420) in foxtail millet isolates and 0.0 to 1.0 (average 0.062) in rice isolates.

3.4. Genetic variability among M. grisea isolates from different hosts

Cluster analysis classified the isolates into three major groups that corresponded with the host specificity of the isolates (Fig. 1). However, there was an exception to this correspondence; two finger millet isolates (FMP1 and FMV20) were placed in

group,otherwiseconstituted by foxtail millet isolates. Overall topology of the dendrogram indicated the presence of three lineages in *M. grisea* species complex infecting different hosts. Several subgroups were observed for populations from finger and foxtail millet indicating high genetic variability within and between different host-limited forms of *M. grisea*. Of the 56 isolates from finger millet, 53 were clustered together in one group, whereas the other 2 were grouped together with foxtail millet isolates,and one isolate (FMP7), althoughsharing slight below 50% similarity was still most closely associated with thefinger millet group.

As all but two of the isolates were clustered in host-specific groups, all the SSR allelic data were inspected to determine host-specific alleles. Three SSR loci (*Pyrms 15-16, Pyrms 37-38, Pyrms 63-64)*showed alleles unique to finger millet-infecting isolates. In terms of locations-specific alleles among the isolates, five SSR loci (*Pyrms 45-46, Pyrms 59-60, Pyrms 61-62, Pyrms 87-88, Pyrms 125-126*) showed unique alleles for the isolates from Mandya, and one SSR marker (*Pyrms 47-48*)detected a unique allele for the isolates from Vizianagaram.

3.5. Analysis of molecular variance (AMOVA)

Analysis of molecular variance (AMOVA) indicated that 52% of the total variation among the isolates used in this study was due to differences between the pathogen populations adapted to different hosts, 42% was due to differences in the isolates from the same host, and the remaining 6% due to heterozygosity within isolates.

3.6. Genetic structure of M. grisea isolates

Analysis of 65 *M. grisea* isolates for population structure using a model-based approach providedevidence for the presence of significant population structure in*M. grisea* and identified two genetically distinct groups or admixtures within the *M. grisea* isolates

from different hosts. The model-based simulation of population structure using SSRs showed the estimated likelihood values being variable among different runs (*K*= 2–15).However, inference of the exact value of *K* (gene pool) was not straightforward because theestimated LnP(D) values increased continuously till*K* = 15 (Fig. 2A), although aplateau started developing at K=8. There were abrupt changes in LnP(D) value between *K* = 5 and *K* = 6; *K* = 6 and*K* = 7;*K* = 7 and *K* = 8. The model choice criterion to detect the most probable value of *K* was Δ*K* (Fig. 2B).The highest value of Δ*K*for this data set was found at*K* = 2 (Fig. 2B). This suggested that the set of isolates was partitioned into two groups (subpopulations), which corresponded to the host origin with a few exceptions (Fig. 3). According to the membership pattern when *K* = 2, group 2 was the largest with 54 (83%) isolates representing only finger millet from different locations. Group 1 was represented by 11 isolates which included all the foxtal millet and rice isolates, and two finger millet isolates (FMP1 and FMV20).

4.0. Discussion

We evaluated 24 SSR markers reported by Kaye et al. [22]for assaying the molecular diversity in *M. grisea* populations adapted to different hosts. The polymorphism detected by selectedSSRs in *M. grisea* was quite high and thus can be used as an efficient tool for genetic diversity studies. The percentage of polymorphic SSRs observed here is very close to that reported by Kaye et al. [22] and by Zheng et al. [23] among *M. grisea* isolates from rice. In contrast, Suzuki et al. [24] observed very low levels of polymorphisms in the *M. grisea* isolates collected in Japan and concluded that the field isolates collected in recent years probably were genetically similar and belonged to a limited number of lineages [31].

The number of alleles per locus in the present study was positively correlated with gene diversity (r = 0.83, P < 0.01) and common alleles (r = 0.98, P<0.01). Positive relationships observed between allele size range and the amount of variation at SSR loci (as

measured by allele/locus and gene diversity) indicated that SSR loci with large allele range show greater variation. It has been suggested that SSR polymorphism results from two different mechanisms: slippage during replication and unequal crossing over [32]. Occurrence of both mating types in *M. grisea* populations infecting finger millet has been reported in India [14]. Therefore,thepolymorphisms detected in our study could havebeen generated both because of unequal crossing over and by replication slippage.The number of repeats of a SSR marker is a useful predictor of its possible polymorphism [33].Wefound that SSRs with longer repeat motifs were less polymorphic (Table 2). Similar observations were madeby Zheng et al. [23] in *M. grisea* populations adapted to rice.

The polymorphic SSR markers in the present study detected 2 to 13 alleles with an average of 6.18 alleles per locus. Variable number of alleles per locus has been reported in previous studies on *M. grisea* populations [22,23,24]. Variation in allele number observed in the present study and that reported in the earlier studies could be due to the large population size and the sampling strategy used to recover isolates in these areas as well as the extent of genetic variation in the isolates[34]. Similarly, variation in the PIC valueswas observed in our study and those reported earlier. The higher gene diversity value in the present study can be attributed to the diverse *M. grisea* isolates collected from different hosts and locations [22]. Nevertheless, the reported PIC values for these SSR primer pairs may be useful in selecting comparatively more informative markers for assessment of molecular diversity in *M. grisea* isolates from India or elsewhere.

We found that the isolates originatingfrom different plant parts (leaf and neck blast) of the same finger millet genotype were randomly distributed in the dendrogram, while some of the isolates from the infected neck and fingers of the same genotypes were grouped in one cluster. These results indicate that multiple independent infections occur on the same plant and an infection may progress to the finger from the neck and *vice versa*. These observations

also indicate that there are no strains specific to leaf, neck or finger blast[35]. In addition, finger millet varieties have shown a consistent reaction to different forms of blast, with limited exceptions [9,36]. Diversity in pathogen populations has also been reported to be higher within field and between cultivars rather than between sub-populations from leaf and panicle in rice [37].

A high degree of variation was observed within the isolates from the same host, especially among isolates from finger millet where a large number of isolates were collected.Several clusters of the isolates from finger millet were observed in the dendrogram depicting genetic variation among the isolates from the same host. Similar results have been documented by Singh and Kumar [7]. In general, isolates from same host were grouped together; however, two finger millet isolates (FMP1 and FMV20) shared SSR profile and clustered along with foxtail millet isolates indicating potential for gene flow occurring between pathogen populations adapted to two different hosts. These findings are in agreement with Rathouret al. [38] who suggested the possibility of gene flow between the *M. grisea*populations infecting finger millet and jungle rice. Evidence also exists for genetic recombination between the *M. grisea* infecting rice and finger millet in the Indian Himalayas [39,40] where both the hosts have been growing sympatrically for centuries. In contrast, Vijiet al. [14] reported that the blast fungus collected from rice and finger millet did not cross-infect and also gave different fingerprint patterns based on MGR-DNA fingerprinting. In the present study, the DNA polymorphism did not reflect the geographical distribution of isolates. Similar observations were reported by Xia et al. [41]for rice blast and Takanet al. [3]for finger millet blast, though in some cases importance of geographical regions has been correlated [42].

An insight into the structure of *M. grisea* populations from different hosts and locations is valuable in enhancing our understanding of the biology of the pathogen and

potentially adaptive genotypic diversity in the species. Model-based population structure analysis of *M. grisea* did not reveal any location/region specific grouping of isolates. However, most of the isolates were grouped based on their host with a few exceptions. All the isolates from rice and foxtail millet were grouped together in Group 1 along with two finger millet isolates (FMP1 and FMV20). Group 2 consisted of mostly genetically similar isolates from finger millet with a few exceptions (Fig. 3) showing some admixture. These included two isolates each from Nandyal (FMNd34 and FMNd48) and Patancheru (FMP7 and FMP12). These differences in population structure among isolates within the same species and geographic regions are likely related to differences in evolutionary history and ecology [34]. Similar observations were made by Tosaet al. [43] who found that *Oryza* and *Setaria* isolates shared two avirulence genes *PWT1* and *PWT2* and were genetically closer to each other.

In finger millet-blast system, resistance breeding has proven to be difficult; however, efforts are being made for the genetic improvement of finger millet especially for blast resistance[3,17]. Present study provides some insight into the biology of *M. grisea* adapted to finger millet and its relationship with the pathogen populations adapted to rice and foxtail millet. The genetic diversity observed in the finger millet adapted populations of *M. grisea* might be indicative of variation for pathogenicity as well. Thus, understanding the pathogenic nature of the populations belonging to different lineages will help forming the framework for finger millet blast management programs especially through host plant resistance.

Acknowledgments

The authors gratefully acknowledge the financial support from the BMZ/GTZ project on "Sustainable conservation and utilization of genetic resources of two underutilized crops-finger millet and foxtail millet-to enhance productivity, nutrition and income in Africa and

Asia" funded by the Federal Ministry for Economic Cooperation and Development (BMZ), Germany to carry out this work.

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Figure captions

Fig. 1. Dendrogram depicting the genetic relationship among 65 isolates of *M. grisea* from different hosts based on SSR data.

Fig. 2.(A) Log-likelihood of the data (n = 65), L (*K*), as a function of *K* (number of groups used to stratify the sample). (B) Values of Δ*K*, with its modal value used to detecttrue *K* of the group (*K* = 2). For each *K* value, at least three independent runs were considered and averaged over the replicates.

Fig. 3. Ancestries of 65 isolates estimated from 17 SSR loci using STRUCTURE version 2.3.4. Different colors represent subpopulations (or groups) in *Magnaporthe grisea* isolates from finger millet, foxtail millet and rice. The height of each bar represents the probability of isolates belonging to different groups. Group 1 included all foxtail millet and rice blast isolates, and two finger millet isolates (FMP1 and FMV20); Group 2 included remaining finger millet isolates.

Table 1

Origin of*Magnaporthe grisea*isolates used in the study.

Identity	Host	Cultivar	Year	Isolated from	Place of collection
FMP1	Finger millet	VL 149	2008	Neck	ICRISAT, Patancheru, Andhra Pradesh
FMP2	Finger millet	VR 708	2009	Neck	ICRISAT, Patancheru, Andhra Pradesh
FMP3	Finger millet	IE 518	2009	Finger	ICRISAT, Patancheru, Andhra Pradesh
FMP4	Finger millet	IE 588	2009	Neck	ICRISAT, Patancheru, Andhra Pradesh
FMP5	Finger millet	IE 2322	2009	Finger	ICRISAT, Patancheru, Andhra Pradesh
FMP6	Finger millet	IE 2323	2009	Finger	ICRISAT, Patancheru, Andhra Pradesh
FMP7	Finger millet	IE 2354	2008	Finger	ICRISAT, Patancheru, Andhra Pradesh
FMP8	Finger millet	IE 2517	2008	Neck	ICRISAT, Patancheru, Andhra Pradesh
FMP9	Finger millet	IE 3038	2009	Neck	ICRISAT, Patancheru, Andhra Pradesh
FMP10	Finger millet	IE 3470	2009	Finger	ICRISAT, Patancheru, Andhra Pradesh
FMP11	Finger millet	IE 4545	2009	Neck	ICRISAT, Patancheru, Andhra Pradesh
FMP12	Finger millet	IE 6154	2009	Finger	ICRISAT, Patancheru, Andhra Pradesh
FMP13	Finger millet	IE 6473	2009	Finger	ICRISAT, Patancheru, Andhra Pradesh
FMV14	Finger millet	VL 149	2009	Neck	ARS, Vizianagaram, Andhra Pradesh
FMV15	Finger millet	PSE 110	2009	Finger	ARS, Vizianagaram, Andhra Pradesh
FMV16	Finger millet	VR 708	2009	Finger	ARS, Vizianagaram, Andhra Pradesh
FMV17	Finger millet	VR 943	2009	Neck	ARS, Vizianagaram, Andhra Pradesh
FMV18	Finger millet	IE 196	2009	Finger	ARS, Vizianagaram, Andhra Pradesh
FMV19	Finger millet	IE 501	2009	Neck	ARS, Vizianagaram, Andhra Pradesh
FMV20	Finger millet	IE 1299	2008	Neck	ARS, Vizianagaram, Andhra Pradesh
FMV21	Finger millet	IE 2322	2009	Neck	ARS, Vizianagaram, Andhra Pradesh
FMV22	Finger millet	IE 3270	2009	Neck	ARS, Vizianagaram, Andhra Pradesh
FMV23	Finger millet	IE 3470	2009	Finger	ARS, Vizianagaram, Andhra Pradesh
FMV24	Finger millet	IE 4750	2009	Leaf	ARS, Vizianagaram, Andhra Pradesh
FMV25	Finger millet	IE 4759	2008	Neck	ARS, Vizianagaram, Andhra Pradesh
FMV26	Finger millet	IE 5736	2009	Neck	ARS, Vizianagaram, Andhra Pradesh

FMNd27	Finger millet	VR 708	2009	Finger	RARS, Nandyal, Andhra Pradesh
FMNd28	Finger millet	IE 501	2009	Neck	RARS, Nandyal, Andhra Pradesh
FMNd29	Finger millet	IE 518	2009	Neck	RARS, Nandyal, Andhra Pradesh
FMNd30	Finger millet	IE 588	2009	Finger	RARS, Nandyal, Andhra Pradesh
FMNd31	Finger millet	IE 3270	2008	Neck	RARS, Nandyal, Andhra Pradesh
FMNd32	Finger millet	IE 3470	2009	Finger	RARS, Nandyal, Andhra Pradesh
FMNd33	Finger millet	IE 4545	2009	Neck	RARS, Nandyal, Andhra Pradesh
FMNd34	Finger millet	IE 5525	2008	Leaf	RARS, Nandyal, Andhra Pradesh
FMNd35	Finger millet	IE 5788	2008	Leaf	RARS, Nandyal, Andhra Pradesh
FMNd36	Finger millet	IE 5843	2008	Leaf	RARS, Nandyal, Andhra Pradesh
FMNd37	Finger millet	IE 6055	2008	Leaf	RARS, Nandyal, Andhra Pradesh
FMNd38	Finger millet	IE 6165	2008	Leaf	RARS, Nandyal, Andhra Pradesh
FMM39	Finger millet	MR 6	2009	Neck	ZARS, Mandya, Karnataka
FMM40	Finger millet	IE 518	2009	Finger	ZARS, Mandya, Karnataka
FMM41	Finger millet	IE 588	2009	Neck	ZARS, Mandya, Karnataka
FMM42	Finger millet	IE 2790	2009	Neck	ZARS, Mandya, Karnataka
FMM43	Finger millet	IE 3470	2009	Finger	ZARS, Mandya, Karnataka
FMM44	Finger millet	IE 5177	2008	Finger	ZARS, Mandya, Karnataka
FMM45	Finger millet	IE 6165	2009	Leaf	ZARS, Mandya, Karnataka
FMM46	Finger millet	IE 6165	2009	Finger	ZARS, Mandya, Karnataka
FMM47	Finger millet	IE 6337	2009	Node	ZARS, Mandya, Karnataka
FMNg48	Finger millet	MR 6	2009	Leaf	OFRS, Naganahalli, Mysore, Karnataka
FMNg49	Finger millet	IE 518	2009	Neck	OFRS, Naganahalli, Mysore, Karnataka
FMNg50	Finger millet	IE 2572	2009	Leaf	OFRS, Naganahalli, Mysore, Karnataka
FMNg51	Finger millet	IE 2572	2009	Neck	OFRS, Naganahalli, Mysore, Karnataka
FMNg52	Finger millet	IE 2572	2009	Finger	OFRS, Naganahalli, Mysore, Karnataka
FMNg53	Finger millet	IE 4545	2009	Neck	OFRS, Naganahalli, Mysore, Karnataka
FMNg54	Finger millet	IE 6154	2009	Leaf	OFRS, Naganahalli, Mysore, Karnataka
FMNg55	Finger millet	IE 6154	2009	Neck	OFRS, Naganahalli, Mysore, Karnataka

FMD56	Finger millet	IE 2857	2008	Neck	RAU, Dholi, Bihar
FxMP57	Foxtail millet	ISe 376	2009	Leaf	ICRISAT, Patancheru, Andhra Pradesh
FxMNd58	Foxtail millet	ISe 1541	2008	Leaf	RARS, Nandyal, Andhra Pradesh.
FxMV59	Foxtail millet	ISe 376	2008	Leaf	ARS, Vizianagaram, Andhra Pradesh
FxMV60	Foxtail millet	ISe 376	2009	Leaf	ARS, Vizianagaram, Andhra Pradesh
FxMM61	Foxtail millet	ISe 376	2009	Leaf	ZARS, Mandya, Karnataka
FxMM62	Foxtail millet	ISe 1541	2009	Leaf	ZARS, Mandya, Karnataka
RM 63	Rice	Vijaya	2009	Leaf	ZARS, Mandya, Karnataka
RM 64	Rice	Vijaya	2010	Leaf	ZARS, Mandya, Karnataka
RM 65	Rice	Vijaya	2010	Leaf	ZARS, Mandya, Karnataka
Pg 21	Pearl millet	Unknown hybrid	2009	Leaf	Farmers field, Jalna, Maharashtra
Pg 37	Pearl millet	Nandi 3	2009	Leaf	Farmers field, Aurangabad, Maharashtra
Pg 39	Pearl millet	ICMB 95222	2009	Leaf	Hissar, Haryana
Pg 41	Pearl millet	ICMB 95444	2009	Leaf	ARS, Durgapura, Jaipur, Rajasthan
Pg 43	Pearl millet	Unknown hybrid	2009	Leaf	Aligarh, Uttar Pradesh
Pg 45	Pearl millet	ICMB 95444	2009	Leaf	ICRISAT, Patancheru, Andhra Pradesh
Pg 118	Pearl millet	Unknown hybrid	2010	Leaf	Rewari, Haryana

ICRISAT: International Crops research Institute for the Semi-Arid Tropics; A.P: Andhra Pradesh; ARS: Agricultural Research Station; RARS: Regional Agricultural Research Station; ZARS: Zonal Agricultural Research Station; OFRS: Organic Farming Research Station

Table 2

Allele composition, polymorphic information content (PIC), gene diversity and heterozygosity (%) of 17 SSR primers in 65 isolates of *M. grisea* from finger millet, foxtail millet and rice.

Marker	Primer sequence (5'→3')	Source	SSR type	Allele composition						M_{AF}	PIC	Gene diversity	Heterozygosity
				Allelic richness	Size range (bp)	Rare (1%)	Common (≤20%)	Frequent (21-50%)	Most frequent (>50%)				
Pyrms 7 and 8	gcaaataacataggaaaacg agaaagagacaaaacactgg	Full BAC (70-15)	$(CT/GA)_{29}$	7	123-179	0	6	-	1	0.600	0.558	0.593	0.000
Pyrms 15 and 16	ttcttccatttctctcgtcttc cgattgtggggtatgtgatag	EST (P12)	$(CT/GA)_{20}$	13	151-200	0	12	1	-	0.379	0.785	0.803	0.031
Pyrms 37 and 38	accctacccccactcatttc aggatcagccaatgccaagt	BAC end (70-15)	$(CA/GT)_6$ + $(CT/GA)_{12}$	2	213-217	0	1	-	1	0.866	0.205	0.232	0.018
Pyrms 41 and 42	aacgtgacaatgtgagcagc gccatgttctaaggtgctgag	BAC end (70-15)	$(CT/GA)_{16}$	6	119-193	1	4	-	1	0.830	0.286	0.300	0.015
Pyrms 45 and 46	ccactttatagcccacccagt ctcttttctcgcaggaggtg	BAC end (70-15)	$(TA/AT)_{11}$	4	214-223	0	2	1	1	0.569	0.473	0.554	0.586
Pyrms 47 and 48	tcacatttgcttgctggagt agacagggttgacggctaaa	BAC end (70-15)	$(TA/AT)_{15}$	6	182-206	0	4	2	-	0.369	0.647	0.700	0.031
Pyrms 59 and 60	ttctcagtaggcttggaattga cttgattggtggtggtgttg	BAC end (70-15)	$(TA/AT)_{12}$	3	183-212	0	2	1	-	0.864	0.217	0.238	0.000
Pyrms 61 and 62	gaggcaacttggcatctacc	BAC end (70-15)	$(GA/CT)_9$	10	230-281	0	9	1	-	0.406	0.760	0.780	0.000

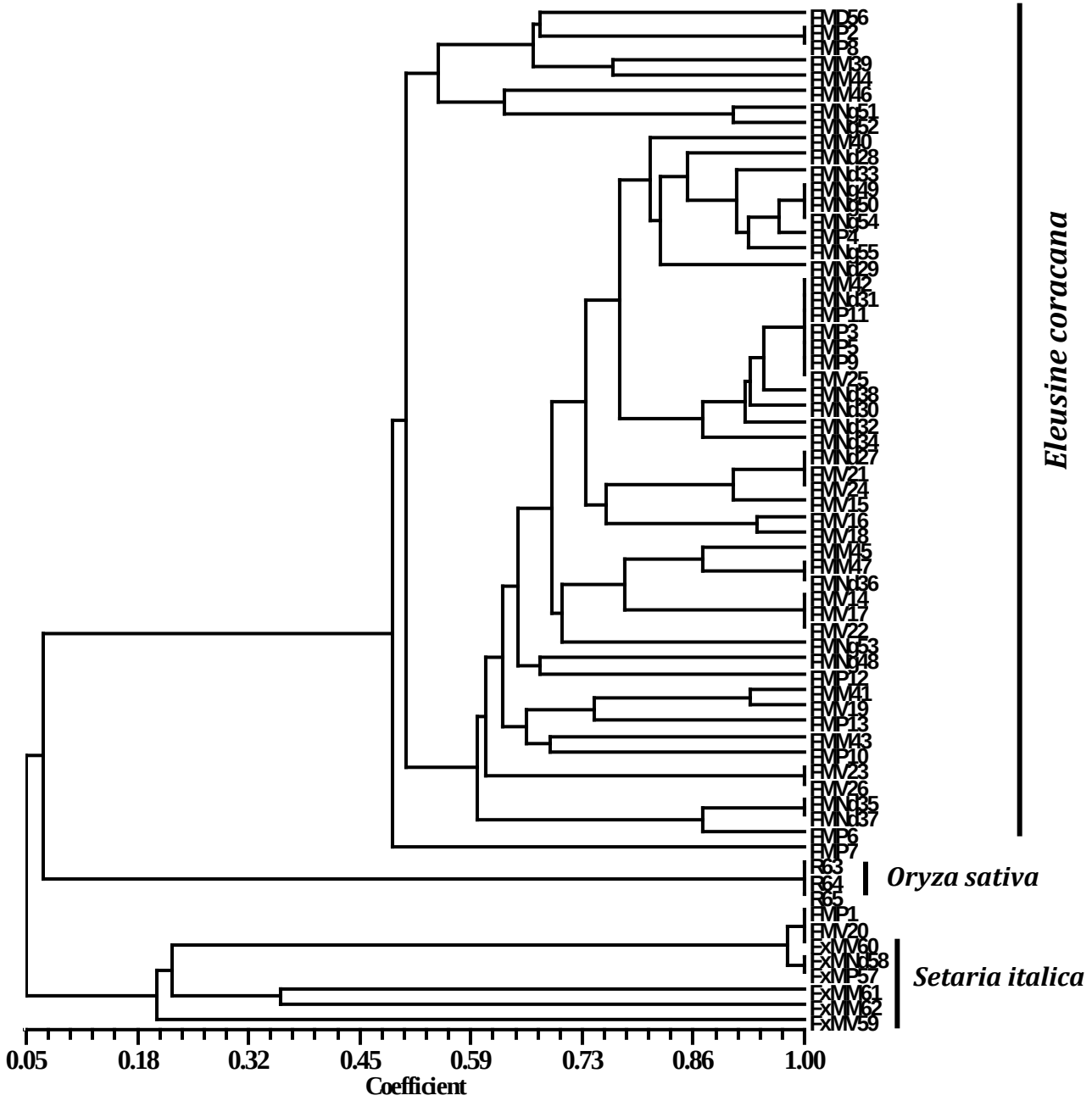
	tggattacagaggcgttcg												
Pyrms 63 and 64	ttgggatcttcggtaagacg gccgacaagacactgaatga	BAC end (70-15)	$(CT/GA)_{15}$	4	169-183	0	3	-	1	0.800	0.316	0.341	0.031
Pyrms 67 and 68	agcaagcaggagatgcagac gtttggctggcaagacagtt	SSR library (Guy11)	$(CA/GT)_{17}$	9	191-233	0	7	2	-	0.246	0.805	0.827	0.046
Pyrms 77 and 78	gaagtattgcacacaaacac gctttcggcaagcctaatc	SSR library (Guy11)	$(CA/GT)_{24}$	8	162-240	0	7	-	1	0.564	0.606	0.636	0.000
Pyrms 87 and 88	Agacttgttactcgggtcttga ccagatgtcactcccctgta	BAC end (70-15)	$(TGC/ACG)_{12}$	4	180-195	0	3	-	1	0.646	0.483	0.529	0.000
Pyrms 93 and 94	Cctcgactccttcaccaaaa cggagagctcaggaagagg	Est (70-15)	$(ATC/TAC)_{12.5}$	5	214-235	0	4	-	1	0.769	0.373	0.392	0.000
Pyrms 99 and 100	Caccactttatggcgcagt acctaggtaggtatacatgttgtt	BAC end (70-15)	$(ACC/TGG)_{20}$	4	195-238	0	3	-	1	0.769	0.357	0.385	0.031
Pyrms 107 and 108	Gcagcaagcagcaatatcag gtggatatcgaaggccaagg	SSR library (Guy11)	$(GA/CT)_{10}$	8	344-384	0	6	1	1	0.592	0.558	0.596	0.015
Pyrms 109 and 110	Tacagtgggagggcaaagag ccagatcgagaaaggggtat	SSR library (Guy11)	$(TG/AC)_{12}$	8	192-225	0	7	-	1	0.562	0.611	0.640	0.016
Pyrms 125 and 126	Ctctccggccaagattga ggttgttgggagaaagaacg	Full BAC (70-15)	$(CAA/GTT)_{32}$	4	133-190	0	3	-	1	0.868	0.225	0.237	0.000
Total				105	-	1	83	9	12	-	-	-	-
Mean				6.18	-	0.05	4.88	0.52	0.70	0.629	0.486	0.517	0.048
Range				2–13	119–384	0–1	1–12	1–2	-	0.246-0.868	0.205–0.805	0.232–0.827	0.000–0.586

Table 3

Summary statistics of 17 SSR markers in 65 isolates of *M. grisea* from finger millet, foxtail millet and rice.

Statistics	Overall	*M. grisea* isolates from		
		Finger millet	Foxtail millet	Rice
Sample size	65	56	6	3
Total number of alleles	105	75	44	15
No. of alleles per locus	6.18 (2-13)	4.41 (2-13)	2.75 (1-4)	0.9
Gene diversity	0.517 (0.232-0.827)	0.402 (0.069-0.790)	0.477 (0-0.667)	0.06 (0-1.0)
Heterozygosity	0.048 (0-0.586)	0.053 (0-0.642)	0.010 (0-0.167)	0
PIC	0.486 (0.205-0.805)	0.369 (0.067-0.759)	0.420 (0-0.620)	0.062 (0-1.00)
Rare alleles	1	1	0	0
Common alleles	83	51	22	0
Frequent alleles	9	10	12	-
Most frequent alleles	12	13	10	15

Figures in parentheses represent range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EMP7
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R64
R65
EMP1
EMV20
ExMM60
ExMN058
ExMP57
ExMM61
ExMM62
ExMM59
Eleusine coracana
Oryza sativa
Setaria italica
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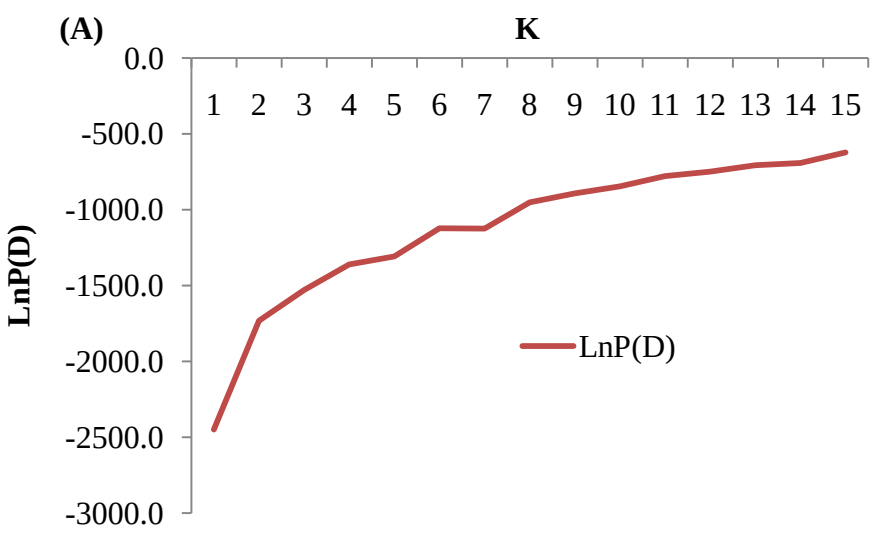
0.45
0.59
0.73
0.86
1.00
Coefficient


(A)
K
0.0
-500.0
-1000.0
-1500.0
-2000.0
-2500.0
-3000.0
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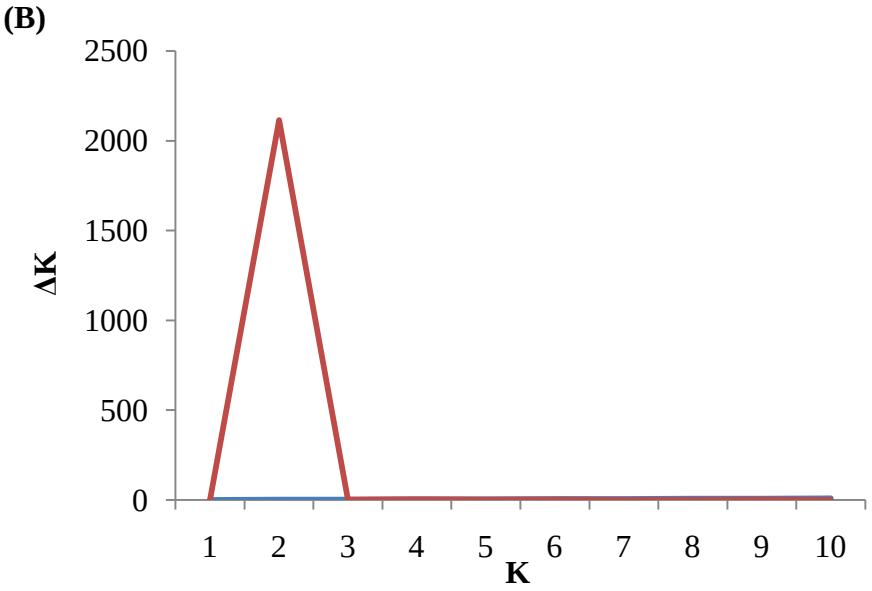
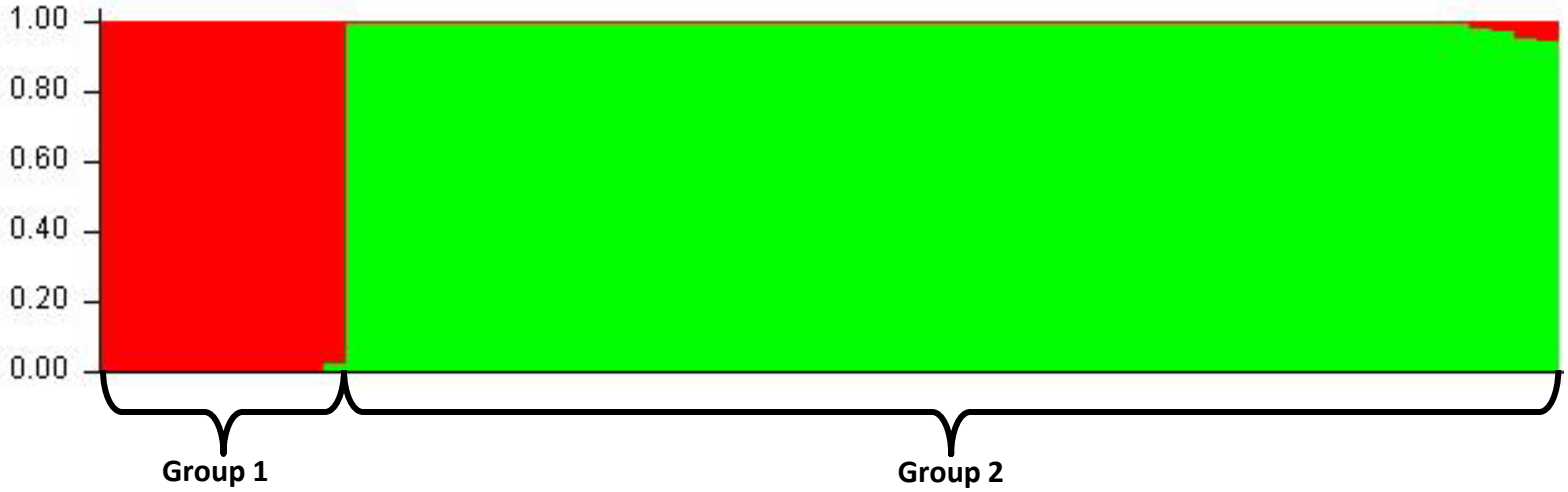
LnP(D)
LnP(D)


(B)
2500
2000
1500
1000
500
0
ΔK
1 2 3 4 5 6 7 8 9 10
K


1.00
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0.20
0.00
Group 1
Group 2