



Identification and Genotyping of SNPs in *RKM1* and *RKM4* Genes of *Sordaria fimicola*

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ABSTRACT

Single nucleotide polymorphisms (SNPs) are one of the most common and abundant class of molecular markers present in the genome of many organisms. The current study represents the first attempt to investigate the natural variations in the *RK-MTases* genes; Ribosomal N-lysine methyltransferase1 (*RKM1*) and Ribosomal N-lysine methyltransferase4 (*RKM4*) in *Sordaria fimicola* using SNP markers. A total seven SNPs in the *RKM1* gene and nine in *RKM4* gene were identified. A subset of SNPs were unique in SFS strains and others were fixed in the NFS strains of *S. fimicola*. These polymorphisms might be adaptive in stressful environmental conditions. Genotyping of eight SNPs of *RK-MTases* genes of *S. fimicola* was accomplished by designing allele specific primers via amplification refractory mutation system-PCR (ARMS-PCR) yielding amplicons of different sizes. This study concluded that SNP markers are an efficient and informative marker system in *S. fimicola*. Most of the studied SNPs are non-synonymous substitutions, which might underpin functional differences in their protein products.

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Authors' Contribution

IM did experimental work and wrote the manuscript. RA and MI Helped in experimental work. SFL reviewed the paper. MS supervised the research.

Key words

Single nucleotide polymorphisms, *RK-MTases*, ARMS-PCR, *Sordaria fimicola*, NFS strains, SFS strains

INTRODUCTION

From last two decades, single nucleotide polymorphisms (SNPs) have become the most popular molecular marker system to study polymorphisms in natural populations of numerous organisms (Väli *et al.*, 2008; Coates *et al.*, 2009; Ljungqvist *et al.*, 2010; Guichoux *et al.*, 2011; Fischer *et al.*, 2017). SNPs are the most abundant type of molecular marker and can be identified in animals, plants and as well as fungi. Their abundance make them ideal for the study of inheritance of genomic regions including exonic and intronic regions (Berger *et al.*, 2001; Wicks *et al.*, 2001; Stickney *et al.*, 2002).

SNP is one of the simplest and most common forms of polymorphism which arises due to the substitution of one nucleotide with the other nucleotide (Shastri, 2002). Due to the environmental stress, gene conversion, and deficiency in the DNA repair mechanisms. These variations are driving force of species evolution and adaptation (Lamb *et al.*, 1998; Hoffmann and Hercus, 2000; Saleem *et al.*, 2001).

Owing to their abundance, SNPs are present in the frequency of approximately one in every kilobase in the

human genome (Brookes, 1999). These simplest forms of genetic variation are more in the non-coding regions, with less deleterious effects. Those SNPs, which do not change the encoded amino acids are known as synonymous substitutions and are usually not involved in natural selection (Kimura, 1983). In contrast, SNPs that alter the encoded amino acids are recognized as non-synonymous substitutions and are more likely to be under natural selection. SNPs can be observed between individuals in a population, may change the promotor activity, influence the DNA and pre mRNA conformation as well as change the phenotypic expression (Lamb *et al.*, 1998; Hoffmann and Hercus, 2000; Saleem *et al.*, 2001).

SNPs are present twice as frequent in non-coding and intergenic regions than in coding regions of the genome (Zhao *et al.*, 2003). Moreover, genome wide studies depict that SNPs of non-coding regions are physically associated with functional regions of genome (Kim *et al.*, 2007). These days, automated next generation sequencing make the SNPs detection and genotyping straightforward (Kaiser *et al.*, 2016). In addition, current SNP based studies generally need to bear high upfront costs in SNP discovery (Chen *et al.*, 2008; Lai *et al.*, 2007) and then genotype them in target organisms (Van Orsouw *et al.*, 2007; Van Tassell *et al.*, 2008).

For low and medium throughputs SNPs genotyping PCR is most commonly used (Chuang *et al.*, 2008). There are many PCR methods available for this purpose but

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selecting the suitable one is critical key factors that taken into account include the nature of polymorphism, number and type of samples, and availability of appropriate instrument for the desired sensitivity and throughput levels (Hamajima *et al.*, 2002).

A very simple and cost-effective method for SNP genotyping is tetra-primer amplification refractory mutation system-PCR (ARMS-PCR), which requires only a PCR reaction followed by gel electrophoresis (Ye *et al.*, 2001). We applied ARMS-PCR approach to study genetic variation in the fungus *Sordaria fimicola*, targeting SNPs in *RKM1* and *RKM4* genes.

MATERIALS AND METHODS

Sub-culturing of fungi

S. fimicola strains (S1, S2, S3, N5, N6 and N7) were sub-cultured on potato dextrose agar (PDA) media under sterile conditions, which were provided by Molecular Genetics Laboratory, University of the Punjab, Lahore. These strains were originally collected from "Evolution Canyon" in Israel (S1, S2 and S3 strains were collected from the South Facing Slope (SFS) and the N5, N6, N7 strains from the North Facing Slope (NFS) of the "Evolution Canyon". The fungal cultures were incubated at 20°C in refrigerated incubator for 7-9 days and were harvested for DNA extraction.

DNA extraction and PCR amplification of *RKM1* and *RKM4* genes

DNA extraction from all studied strains of *S. fimicola* was carried out by using modified DNA extraction protocol of Pietro *et al.* (1995). Four primer pairs (two for each gene) were used to amplify both *RK-MTases* genes. The primer pairs used for amplification of *RKM1* gene were; RKM1F1 (5'-GTAAAAGCACTACTTCAGT-3'), RKM1R1 (5'-ACAAATCCATATCCAGAGAG-3') and RKM1F2 (5'-TAAATTGCCATTAGATGTGG-3'), RKM1R2 (5'-TAAAATAGTCTCTTCGGTTG-3').

For *RKM4* gene, primer pairs were; RKM4F1 (5'-AGAGATACCGAAAACCTTTGT-3'), RKM4R1 (5'-CAGTTAGAGTCGTAAGTTAA-3') and RKM4F2 (5'-GAATGAACAAGTGTACAACA-3'), RKM4R2 (5'-GGACGTTTGACAGAGCTTTT-3'). The PCR reaction volume was 15µl, which contained; 10µl 2X Amp Master Mix (GeneAll), 1µl forward primer, 1µl reverse primer (100µM each), 2µl DNA sample (1 in 10 dilution of the g-DNA stock) and 1µl dd H₂O. Touch down PCR conditions were used to amplify the *RK-MTases* genes. The stage 1 included the 15 cycles with initial denaturation at 95°C for 3 min, second denaturation for 30 sec, annealing at Tm+10°C for 45 sec and elongation

at 72°C for 60 sec. The stage 2 contained 25 cycles with denaturation at 95°C for 30 sec, annealing at Tm-5°C for 45 sec and elongation at 72°C for 60 sec. The termination stage contained elongation at 72°C for 5 min, stop reaction at 4°C for 15 min and final hold at 23°C until removed from thermal cycler. 1.0% agarose gel electrophoresis was carried out to resolve the PCR products, stained with ethidium bromide and visualized under UV light in Gel Documentation System (Syngene).

Sequencing of genes and sequence analysis for SNPs

Amplicons were sequenced at Macrogen Korea and sequences were edited using the BioEdit program. Multiple sequence alignment was carried out for both methyltransferase genes (*RKM1* and *RKM4*) separately using the Clustal Omega online tool (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) to identify SNPs using *S. cerevisiae* sequence as reference.

Designing of primers for SNP sites

For SNP genotyping, a total of eight specific primers based on SNP sites for both *RKM1* and *RKM4* genes were designed; rkm1F1 (specific for SFS strains), rkm1F2 (specific for all strains of *S. fimicola*), rkm1F3 (specific for SFS strains), rkm1F4 (specific for all strains of *S. fimicola*) and rkm4F1, rkm4F2, rkm4F4 (specific for SFS strains), rkm4F3 (specific for NFS strains). The reverse primer RKM1R1 (5'-ACAAATCCATATCCAGAGAG-3'), (specific for *RKM1* gene) was used in combination with the forward primers specific to the SNPs of *RKM1* gene. Likewise, reverse primer RKM4R1 (5'-CAGTTAGAGTCGTAAGTTAA-3'), (specific for *RKM4* gene) was used in combination with the forward primers of SNPs of *RKM4* gene. An additional mismatch at third base towards the 3' end was deliberately introduced in each SNP primer, a G was substituted with a T and a C substituted with an A and vice versa (Table 1).

ARMS PCR conditions for amplification of SNP sites

To differentiate between the target SNPs in the *RKM1* and *RKM4* genes ARMS-PCR conditions were used. For amplification of four SNPs of *RKM1* gene of *S. fimicola*, four forward primers (rkm1F1, rkm1F2, rkm1F3, rkm1F4) along with reverse primer rkm1R1 (specific to *RKM1* gene) were used. Likewise, for four SNPs of *RKM4* gene of *S. fimicola*, four forward primers (rkm4F1, rkm4F2, rkm4F3, rkm4F4) along with reverse primer rkm4R1 (specific to *RKM4* gene) were used. The 20µl PCR reaction mixture for SNPs of *RKM1* gene contained 2µl DNA (1 in 10 dilution of the g-DNA stock), 1µl rkm1F1 primer, 1µl rkm1F2 primer, 1µl rkm1F3 primer, 1µl rkm1F4 primer, 1µl rkm1R1 primer (100µM each), 10µl 2X Amp Master

Mix (GeneAll) and 3µl ddH₂O. The reaction mixture for SNPs of *RKM4* gene was prepared in the same way as for SNPs of *RKM1*. The PCR conditions for SNPs of both genes consisted of 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 62.5 °C, 40 s at 72 °C, and a final 7 min extension at 72 °C (Yang *et al.*, 2017).

Table I. Oligonucleotide primers used in the current study.

Name of primer	Oligonucleotide sequence	Position in gene
RKM1F1	5'-GTTAAAAGCACTACTTCAGT-3'	15-34
RKM1R1	5'-ACAAATCCATATCCAGAGAG-3'	785-804
RKM1F2	5'-TAAATTGCCATTAGATGTGG-3'	844-864
RKM1R2	5'-TAAAATAGTCTCTTCGGTTG-3'	1662-1681
RKM4F1	5'-AGAGATACCGAAAACCTTTGT-3'	16-35
RKM4R1	5'-CAGTTAGAGTCGTAAGTTAA-3'	656-675
RKM4F2	5'-GAATGAACAAGTGTAACAACA-3'	702-727
RKM4R2	5'-GGACGTTTGACAGAGCTTTT-3'	1391-1410
rkm1F1	5'-GTGAATCCACTAAGACT C -3' A	20-37
rkm1F2	5'-AAAGAGTGGTTTGAAT T -3' C	256-273
rkm1F3	5'-TTTATGGTGCACCGT G -3' T	428-445
rkm1F4	5'-GAAACTGTCCTGACACC C -3' A	485-502
rkm4F1	5'-TA A CTGTATACTTTAG G -3' T	33-50
rkm4F2	5'-TAGCGACGTCCTTCG C A-3' A	266-243
rkm4F3	5'-GATTCCACTTGCTGAT A -3' A	424-441
rkm4F4	5'-ATGGTTGCTTTGAG G T A C-3' G	502-519

Note: Bold underlined nucleotides are showing additional mismatches, where G substituted with T and C substituted with A and vice versa. Highlighted nucleotides are showing SNPs.

RESULTS AND DISCUSSION

Molecular markers have become a popular tool for observing polymorphism in plants, animals and fungi. Among all marker systems, SNPs are the most prevalent molecular marker for describing genetic variation in natural populations. These are useful for observing genetic variation, population genetic structure and reconstructing the evolutionary history of species (Banke and McDonald, 2005; Coates *et al.*, 2009; Fischer *et al.*, 2017).

To the best of our knowledge, it is the first time SNPs identified and genotyped in the *RK-MTases* genes in *S. fomicola*. A lot of work has been carried out on SNPs of plants and humans but a very few or negligible studies are done on fungal SNPs. In the present study, SNPs genotyping was carried out by performing allele specific PCR conditions to observe polymorphisms in *RKM1*

and *RKM4* methyltransferase genes of *S. fomicola*. The *RKM1* and *RKM4* regions of *S. fomicola* were amplified with target-specific primers by using touchdown PCR conditions and the product sizes were 1320bp and 900bp respectively.

The results of multiple sequence alignment showed that the *RKM1* and *RKM4* regions for six strains of *S. fomicola* and *S. cerevisiae* were identical except for polymorphic sites. These polymorphic sites are due to substitution of single nucleotide and hence termed as single nucleotide polymorphism (SNP). Total seven SNP sites for *RKM1* region and nine SNP sites for *RKM4* region of *S. fomicola* were identified (Supplementary Figs. 1 and 3) but genotyping of total eight SNPs were carried out for both *RK-MTases* in this study (Figs. 1 and 2). All observed SNPs of *RKM1* region for all strains of *S. fomicola* were identical except for two SNPs, which are unique for SFS strains (Fig. 1). In *RKM4* region, some SNPs are present in SFS strains but not present in NFS strains and vice versa. For example, SNP at 35th position in the *RKM4* region is present only in SFS strains but not present in NFS strains (Fig. 2).

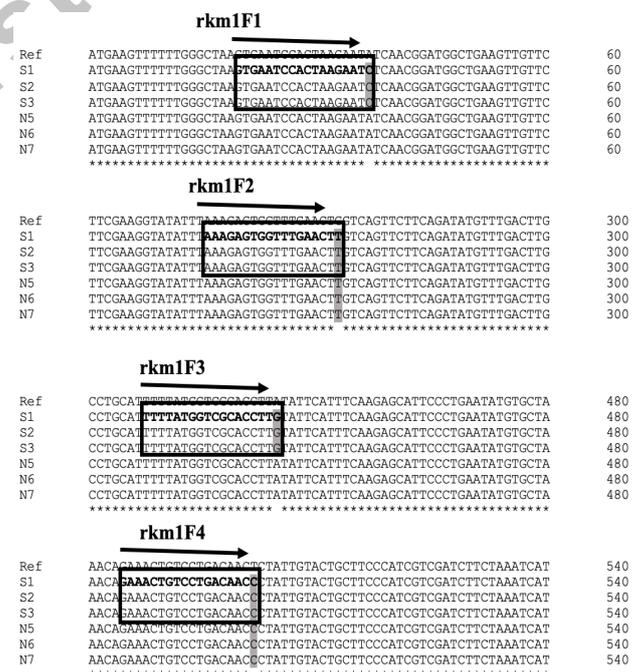


Fig. 1. Multiple sequence alignment of *RKM1* region of NFS and SFS strains of *S. fomicola* with respect to the *Saccharomyces cerevisiae*. Symbol (*) showing conserved sites, space and highlighted regions showing SNPs. Four primers specific to SNP sites are; rkm1F1, rkm1F2, rkm1F3, rkm1F4.

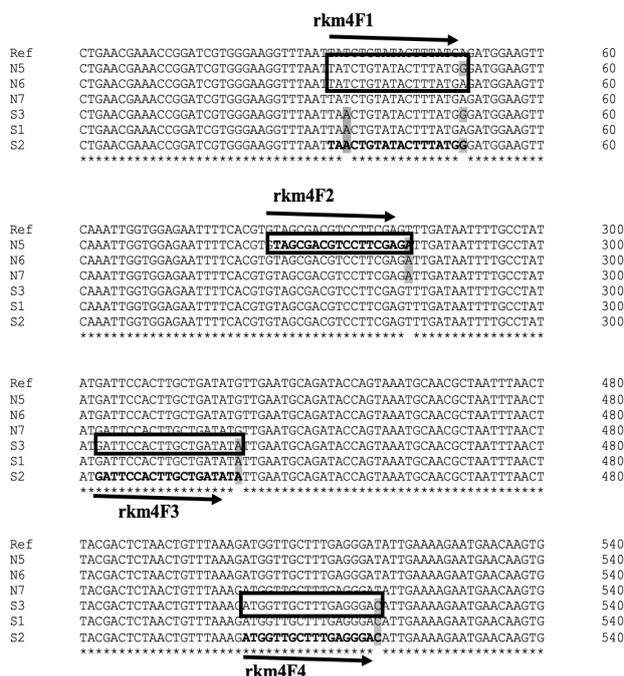


Fig. 2. Multiple sequence alignment of *RKM4* region of *S. fmicola* strains with respect to *S. cerevisiae* in order to observe single nucleotide polymorphism (SNP). Symbol (*) is showing fully conserved sites, space and highlighted regions showing SNPs. Four primers specific to SNP sites are; rkm4F1, rkm4F2, rkm4F3, rkm4F4.

For *RKM4* region, the SNP site at 50th nucleotide position is unique for S1 and S3 strains where A substituted with G. The SNP site at 284th position is unique for NFS strains (T substituted with A) and absent in SFS strains. The SNPs at 442 and 520 nucleotide position, in which G substituted with A and T with C are present only in SFS strains, but not in NFS strains (Fig. 2).

In *RKM1* gene, at first SNP of SFS strains, substitution at first base of codon took place, where A replaced with C (ATC-CTC) which changed the encoded amino acid from isoleucine (I) to leucine (L). In 2nd SNP of SFS and NFS strains, substitution at third base of the codon did not change the encoded amino acid. At 3rd SNP of SFS strains, replacement of A with G, resulted in the change of codon from ATA to GTA and amino acid from isoleucine (I) to valine (V). At 4th SNP of SFS and NFS strains, substitution at first base of codon occurred and codon changed from TCT to CCT and encoded amino acid from serine (S) to proline (P) (Fig. 1 and Supplementary Fig. 2). First and third SNPs of SFS strains showed conservation among the groups of strongly similar properties. These are shown by symbol (:) in the amino acid sequence of RKM1 protein in Supplementary Figure 2.

In *RKM4* gene, at first polymorphic site of SFS strains, T substituted with A at second base of codon, resulted in change of ATC codon into AAC, which changed the Isoleucine (I) into asparagine (N). At second polymorphic site in S2, S3 and N5 strains, A substituted with G, resulting into the change of codon from GAG to GGG, changed the encoding amino acid from glutamate (E) to glycine (G). At 3rd polymorphic site in NFS strains, T replaced with A at first base of the codon, where TTT converted into ATT and changed the amino acid from phenylalanine (F) to isoleucine (I). In SFS strains at 4th polymorphic site, G substituted with A at third base of the codon (ATG-ATA), resulted into the change of methionine (M) into isoleucine (I) (Fig. 2 and Supplementary Fig. 4).

A number of studies have been carried out on SNPs in different genes as well as whole genome studies by next generation sequencing to observe polymorphism in different plants and fungi. Sun *et al.* (2013) identified three SNP rich genomic regions and observed polymorphisms in rice false smut *Ustilaginoidea virens*. Whole genome scan for SNP identification was carried out in Soybean. Likewise, Trick *et al.* (2009) and Park *et al.* (2010) observed SNPs in the whole genome of Brassica and Li *et al.* (2009) reported SNPs in candidate genes controlling morphological traits of leaves and flowering time. Lopez *et al.* (2000) reported SNPs in candidate genes delta 12 fatty acid desaturase and in fatty acid desaturase 2A in *Arachis hypogaea* L.

ARMS has become a standard technique that was first described by Newton and colleagues in 1989. It allows the discrimination of alleles that differ by as little as 1bp. In order to genotype the SNPs, the critical part of ARMS-PCR is to design the primers. A single mismatch at 3' end is not sufficient to avoid non-specific binding, so an extra mismatch was introduced at the 3rd base pair at 3' end to allow specific binding (Wang *et al.*, 2010; Medrano and de oliveiro, 2014).

In the current study, eight SNP sites for both *RKM1* and *RKM4* regions (4 for each) were amplified using SNPs site specific primers. Four forward primers; rkm1F1, rkm1F2, rkm1F3 and rkm1F4 were used in combination with reverse primer RKM1R1 to amplify the four SNP sites of the *RKM1* region. Four SNP sites having 650bp, 520bp, 240bp and 200bp were amplified by ARMS-PCR respectively, shown in schematic diagram in Figure 3a. Likewise, four SNPs primers (rkm4F1, rkm4F2, rkm4F3 and rkm4F4) in combination with reverse primer RKM4R1 used to amplify the SNPs of *RKM4* region and amplicons obtained as; 600bp, 450bp, 250bp and 200bp respectively, shown in schematic diagram in Figure 3b.

ARMS-PCR is allele specific PCR, which is much reliable and reproducible. It does not require restriction

digestion and sequencing of PCR product. It only requires the separation of different DNA fragments by using agarose gel electrophoresis. Hence, it is cost effective technique for genotyping of SNPs in coding and non-coding regions of plants, animals and fungal genome.

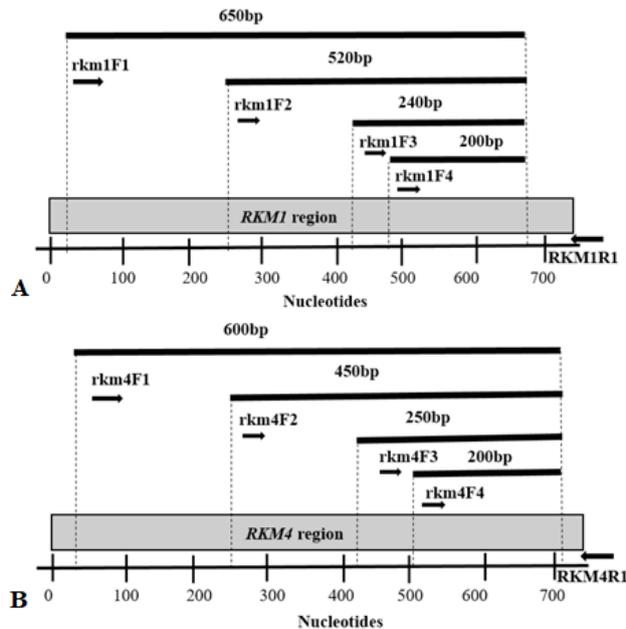


Fig. 3. Schematic diagram of *RKM1* (A) and *RKM4* (B) regions of *S. fimicola* showing primers specific to SNP sites to perform multiplex PCR reaction. rkm1F1, rkm1F2, rkm1F3 and rkm1F4 are specific for SNPs of NFS and SFS strains, while RKM1R1 is reverse primer specific for *RKM1* region.

CONCLUSION

SNP is a reliable, efficient and highly reproducible molecular marker to observe polymorphisms in coding regions as well as in non-coding regions. In this study, we successfully identified and genotyped SNPs in the fungus *S. fimicola* via ARMS-PCR. SNPs specific to the SFS strains and the NFS strains of *S. fimicola* were found, some of which were non-synonymous substitutions, which might have important role in evolution and adaptive values in their respective environmental conditions.

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Supplementary material

There is supplementary material associated with

this article. Access the material online at: <https://dx.doi.org/10.17582/journal.pjz/20190902090906>

Statement of conflict of interest

The authors have declared no conflict of interest.

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Online First Article



Supplementary Material

Identification and Genotyping of SNPs in *RKM1* and *RKM4* Genes of *Sordaria fimicola*

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Ref	ATGAAGTTTTTTGGGCTAAGTGAATCCAATRAAGATATCAACGGATGCGTGAAGTTGTTTC	60	N6	CCTGCATTTTTATGGTGGCACTTATATTTCATTTCAGAGCATTCCCTGGAATATGTGTGA	480
S1	ATGAAGTTTTTTGGGCTAAGTGAATCCAATRAAGATATCAACGGATGCGTGAAGTTGTTTC	60	N7	CCTGCATTTTTATGGTGGCACTTATATTTCATTTCAGAGCATTCCCTGGAATATGTGTGA	480
S2	ATGAAGTTTTTTGGGCTAAGTGAATCCAATRAAGATATCAACGGATGCGTGAAGTTGTTTC	60		*****	
S3	ATGAAGTTTTTTGGGCTAAGTGAATCCAATRAAGATATCAACGGATGCGTGAAGTTGTTTC	60		*****	
N5	ATGAAGTTTTTTGGGCTAAGTGAATCCAATRAAGATATCAACGGATGCGTGAAGTTGTTTC	60		*****	
N6	ATGAAGTTTTTTGGGCTAAGTGAATCCAATRAAGATATCAACGGATGCGTGAAGTTGTTTC	60		*****	
N7	ATGAAGTTTTTTGGGCTAAGTGAATCCAATRAAGATATCAACGGATGCGTGAAGTTGTTTC	60		*****	
Ref	TTCCGAAAAATAAAATTTGATAGGATAATGATACTATCSTGGACAATGTCGTGTGAAT	120	N5	AACGAGAACTGTCTGACAACTCTATTGTACTGCTTCCCATCGTCGATCTTCTAAATCAT	540
S1	TTCCGAAAAATAAAATTTGATAGGATAATGATACTATCSTGGACAATGTCGTGTGAAT	120	N6	AACGAGAACTGTCTGACAACTCTATTGTACTGCTTCCCATCGTCGATCTTCTAAATCAT	540
S2	TTCCGAAAAATAAAATTTGATAGGATAATGATACTATCSTGGACAATGTCGTGTGAAT	120	N7	AACGAGAACTGTCTGACAACTCTATTGTACTGCTTCCCATCGTCGATCTTCTAAATCAT	540
S3	TTCCGAAAAATAAAATTTGATAGGATAATGATACTATCSTGGACAATGTCGTGTGAAT	120		*****	
N5	TTCCGAAAAATAAAATTTGATAGGATAATGATACTATCSTGGACAATGTCGTGTGAAT	120		*****	
N6	TTCCGAAAAATAAAATTTGATAGGATAATGATACTATCSTGGACAATGTCGTGTGAAT	120		*****	
N7	TTCCGAAAAATAAAATTTGATAGGATAATGATACTATCSTGGACAATGTCGTGTGAAT	120		*****	
Ref	GATAAATTTAAACCTTACTTGGATGCATGTCCTCCCGCTAAATTCGCGCTTGGTCTGG	180	S1	GACTACCGTCCCAAAGTCAAATGGTATCCCTGAAATGGTGGTCTCTGTATGAAAAATC	600
S1	GATAAATTTAAACCTTACTTGGATGCATGTCCTCCCGCTAAATTCGCGCTTGGTCTGG	180	S2	GACTACCGTCCCAAAGTCAAATGGTATCCCTGAAATGGTGGTCTCTGTATGAAAAATC	600
S2	GATAAATTTAAACCTTACTTGGATGCATGTCCTCCCGCTAAATTCGCGCTTGGTCTGG	180	S3	GACTACCGTCCCAAAGTCAAATGGTATCCCTGAAATGGTGGTCTCTGTATGAAAAATC	600
S3	GATAAATTTAAACCTTACTTGGATGCATGTCCTCCCGCTAAATTCGCGCTTGGTCTGG	180	N5	GACTACCGTCCCAAAGTCAAATGGTATCCCTGAAATGGTGGTCTCTGTATGAAAAATC	600
N5	GATAAATTTAAACCTTACTTGGATGCATGTCCTCCCGCTAAATTCGCGCTTGGTCTGG	180	N6	GACTACCGTCCCAAAGTCAAATGGTATCCCTGAAATGGTGGTCTCTGTATGAAAAATC	600
N6	GATAAATTTAAACCTTACTTGGATGCATGTCCTCCCGCTAAATTCGCGCTTGGTCTGG	180	N7	GACTACCGTCCCAAAGTCAAATGGTATCCCTGAAATGGTGGTCTCTGTATGAAAAATC	600
N7	GATAAATTTAAACCTTACTTGGATGCATGTCCTCCCGCTAAATTCGCGCTTGGTCTGG	180		*****	
Ref	AACCCAAAGCGAGTTGAAGCGTTTATCATCTCAACAACATAGGGAATTCGATTCATGAAAAG	240	S1	GGTACCGCTCCCAATCAAGAGAACTCAATAAATTTGGCGTAAAGGAAATGAGGAG	660
S1	AACCCAAAGCGAGTTGAAGCGTTTATCATCTCAACAACATAGGGAATTCGATTCATGAAAAG	240	S2	GGTACCGCTCCCAATCAAGAGAACTCAATAAATTTGGCGTAAAGGAAATGAGGAG	660
S2	AACCCAAAGCGAGTTGAAGCGTTTATCATCTCAACAACATAGGGAATTCGATTCATGAAAAG	240	S3	GGTACCGCTCCCAATCAAGAGAACTCAATAAATTTGGCGTAAAGGAAATGAGGAG	660
S3	AACCCAAAGCGAGTTGAAGCGTTTATCATCTCAACAACATAGGGAATTCGATTCATGAAAAG	240	N5	GGTACCGCTCCCAATCAAGAGAACTCAATAAATTTGGCGTAAAGGAAATGAGGAG	660
N5	AACCCAAAGCGAGTTGAAGCGTTTATCATCTCAACAACATAGGGAATTCGATTCATGAAAAG	240	N6	GGTACCGCTCCCAATCAAGAGAACTCAATAAATTTGGCGTAAAGGAAATGAGGAG	660
N6	AACCCAAAGCGAGTTGAAGCGTTTATCATCTCAACAACATAGGGAATTCGATTCATGAAAAG	240	N7	GGTACCGCTCCCAATCAAGAGAACTCAATAAATTTGGCGTAAAGGAAATGAGGAG	660
N7	AACCCAAAGCGAGTTGAAGCGTTTATCATCTCAACAACATAGGGAATTCGATTCATGAAAAG	240		*****	
Ref	TTCCGAGGTATATTTAAAGAGTGGTTGAAGTGGTCAAGTCTTCCAGATATGTTGACTTG	300	S1	TTACTCTCTGGATATGGATTTGTTTTAGAAGACAACATATTTGACTCAGTGGCTTTGAAA	720
S1	TTCCGAGGTATATTTAAAGAGTGGTTGAAGTGGTCAAGTCTTCCAGATATGTTGACTTG	300	S2	TTACTCTCTGGATATGGATTTGTTTTAGAAGACAACATATTTGACTCAGTGGCTTTGAAA	720
S2	TTCCGAGGTATATTTAAAGAGTGGTTGAAGTGGTCAAGTCTTCCAGATATGTTGACTTG	300	S3	TTACTCTCTGGATATGGATTTGTTTTAGAAGACAACATATTTGACTCAGTGGCTTTGAAA	720
S3	TTCCGAGGTATATTTAAAGAGTGGTTGAAGTGGTCAAGTCTTCCAGATATGTTGACTTG	300	N5	TTACTCTCTGGATATGGATTTGTTTTAGAAGACAACATATTTGACTCAGTGGCTTTGAAA	720
N5	TTCCGAGGTATATTTAAAGAGTGGTTGAAGTGGTCAAGTCTTCCAGATATGTTGACTTG	300	N6	TTACTCTCTGGATATGGATTTGTTTTAGAAGACAACATATTTGACTCAGTGGCTTTGAAA	720
N6	TTCCGAGGTATATTTAAAGAGTGGTTGAAGTGGTCAAGTCTTCCAGATATGTTGACTTG	300	N7	TTACTCTCTGGATATGGATTTGTTTTAGAAGACAACATATTTGACTCAGTGGCTTTGAAA	720
N7	TTCCGAGGTATATTTAAAGAGTGGTTGAAGTGGTCAAGTCTTCCAGATATGTTGACTTG	300		*****	
Ref	GAAGAGTGGCAGATGATGTCGAGACTTCCCAATATCTCGATGAGTTGACATATGAGGCT	360	S1	GTAAATGGCCATTAGATGTGGTATCTACAATCTTGAACAAGAACCTAGTTTGAAGCTG	780
S1	GAAGAGTGGCAGATGATGTCGAGACTTCCCAATATCTCGATGAGTTGACATATGAGGCT	360	S2	GTAAATGGCCATTAGATGTGGTATCTACAATCTTGAACAAGAACCTAGTTTGAAGCTG	780
S2	GAAGAGTGGCAGATGATGTCGAGACTTCCCAATATCTCGATGAGTTGACATATGAGGCT	360	S3	GTAAATGGCCATTAGATGTGGTATCTACAATCTTGAACAAGAACCTAGTTTGAAGCTG	780
S3	GAAGAGTGGCAGATGATGTCGAGACTTCCCAATATCTCGATGAGTTGACATATGAGGCT	360	N5	GTAAATGGCCATTAGATGTGGTATCTACAATCTTGAACAAGAACCTAGTTTGAAGCTG	780
N5	GAAGAGTGGCAGATGATGTCGAGACTTCCCAATATCTCGATGAGTTGACATATGAGGCT	360	N6	GTAAATGGCCATTAGATGTGGTATCTACAATCTTGAACAAGAACCTAGTTTGAAGCTG	780
N6	GAAGAGTGGCAGATGATGTCGAGACTTCCCAATATCTCGATGAGTTGACATATGAGGCT	360	N7	GTAAATGGCCATTAGATGTGGTATCTACAATCTTGAACAAGAACCTAGTTTGAAGCTG	780
N7	GAAGAGTGGCAGATGATGTCGAGACTTCCCAATATCTCGATGAGTTGACATATGAGGCT	360		*****	
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S1	TTGTATGAGAGATTTTGAAGATACAGAACTTCAAGACCAACTATCTGGTATTCCTTT	420	S2	CCCTTACTTCGGATTACACCATTATGCTTTGAAAAAAGAACTGTGTCCAGCAAGAA	840
S2	TTGTATGAGAGATTTTGAAGATACAGAACTTCAAGACCAACTATCTGGTATTCCTTT	420	S3	CCCTTACTTCGGATTACACCATTATGCTTTGAAAAAAGAACTGTGTCCAGCAAGAA	840
S3	TTGTATGAGAGATTTTGAAGATACAGAACTTCAAGACCAACTATCTGGTATTCCTTT	420	N5	CCCTTACTTCGGATTACACCATTATGCTTTGAAAAAAGAACTGTGTCCAGCAAGAA	840
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N6	TTGTATGAGAGATTTTGAAGATACAGAACTTCAAGACCAACTATCTGGTATTCCTTT	420	N7	CCCTTACTTCGGATTACACCATTATGCTTTGAAAAAAGAACTGTGTCCAGCAAGAA	840
N7	TTGTATGAGAGATTTTGAAGATACAGAACTTCAAGACCAACTATCTGGTATTCCTTT	420		*****	
Ref	CTCGCATTTTTATGGTCGACCTTATATTCATTTCAAGAGCAATCCCTGAAATATGAGGCTA	480	S1	AAGAAGGCTACTCGTAGTCTACAGACTACATCAATGGGGTGACTTACTTCAATTAACATA	900
S1	CTCGCATTTTTATGGTCGACCTTATATTCATTTCAAGAGCAATCCCTGAAATATGAGGCTA	480	S2	AAGAAGGCTACTCGTAGTCTACAGACTACATCAATGGGGTGACTTACTTCAATTAACATA	900
S2	CTCGCATTTTTATGGTCGACCTTATATTCATTTCAAGAGCAATCCCTGAAATATGAGGCTA	480	S3	AAGAAGGCTACTCGTAGTCTACAGACTACATCAATGGGGTGACTTACTTCAATTAACATA	900
S3	CTCGCATTTTTATGGTCGACCTTATATTCATTTCAAGAGCAATCCCTGAAATATGAGGCTA	480	N5	AAGAAGGCTACTCGTAGTCTACAGACTACATCAATGGGGTGACTTACTTCAATTAACATA	900
N5	CTCGCATTTTTATGGTCGACCTTATATTCATTTCAAGAGCAATCCCTGAAATATGAGGCTA	480	N6	AAGAAGGCTACTCGTAGTCTACAGACTACATCAATGGGGTGACTTACTTCAATTAACATA	900
N6	CTCGCATTTTTATGGTCGACCTTATATTCATTTCAAGAGCAATCCCTGAAATATGAGGCTA	480	N7	AAGAAGGCTACTCGTAGTCTACAGACTACATCAATGGGGTGACTTACTTCAATTAACATA	900
N7	CTCGCATTTTTATGGTCGACCTTATATTCATTTCAAGAGCAATCCCTGAAATATGAGGCTA	480		*****	

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phdgenetics@gmail.com

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Ref	CAAAATGAACAATGTTTAGAACCACTTGGATCTTTTACCTACCTTTCTAAGGCCGAA	960	Ref	MRFFGLSESTRNINWGLKLFPAKIKFRDRNDTIVDNVVRNDRFKFPVLDALPRLNSPLW	60
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S2	CAAAATGAACAATGTTTAGAACCACTTGGATCTTTTACCTACCTTTCTAAGGCCGAA	960	S2	MRFFGLSESTRNINWGLKLFPAKIKFRDRNDTIVDNVVRNDRFKFPVLDALPRLNSPLW	60
S3	CAAAATGAACAATGTTTAGAACCACTTGGATCTTTTACCTACCTTTCTAAGGCCGAA	960	S3	MRFFGLSESTRNINWGLKLFPAKIKFRDRNDTIVDNVVRNDRFKFPVLDALPRLNSPLW	60
N5	CAAAATGAACAATGTTTAGAACCACTTGGATCTTTTACCTACCTTTCTAAGGCCGAA	960	N5	MRFFGLSESTRNINWGLKLFPAKIKFRDRNDTIVDNVVRNDRFKFPVLDALPRLNSPLW	60
N6	CAAAATGAACAATGTTTAGAACCACTTGGATCTTTTACCTACCTTTCTAAGGCCGAA	960	N6	MRFFGLSESTRNINWGLKLFPAKIKFRDRNDTIVDNVVRNDRFKFPVLDALPRLNSPLW	60
N7	CAAAATGAACAATGTTTAGAACCACTTGGATCTTTTACCTACCTTTCTAAGGCCGAA	960	N7	MRFFGLSESTRNINWGLKLFPAKIKFRDRNDTIVDNVVRNDRFKFPVLDALPRLNSPLW	60
Ref	GAGGAGGATCTACAGATTGAGAGCCCGTTTGCAGGGTATACAAATGCTACGAAATGCA	1020	Ref	NPSELKRLSSTNIGNSIHEKFEFIFKFEWFEVSSSDMFLERLVDVQTFHNLDELTYEA	120
S1	GAGGAGGATCTACAGATTGAGAGCCCGTTTGCAGGGTATACAAATGCTACGAAATGCA	1020	S1	NPSELKRLSSTNIGNSIHEKFEFIFKFEWFEVSSSDMFLERLVDVQTFHNLDELTYEA	120
S2	GAGGAGGATCTACAGATTGAGAGCCCGTTTGCAGGGTATACAAATGCTACGAAATGCA	1020	S2	NPSELKRLSSTNIGNSIHEKFEFIFKFEWFEVSSSDMFLERLVDVQTFHNLDELTYEA	120
S3	GAGGAGGATCTACAGATTGAGAGCCCGTTTGCAGGGTATACAAATGCTACGAAATGCA	1020	S3	NPSELKRLSSTNIGNSIHEKFEFIFKFEWFEVSSSDMFLERLVDVQTFHNLDELTYEA	120
N5	GAGGAGGATCTACAGATTGAGAGCCCGTTTGCAGGGTATACAAATGCTACGAAATGCA	1020	N5	NPSELKRLSSTNIGNSIHEKFEFIFKFEWFEVSSSDMFLERLVDVQTFHNLDELTYEA	120
N6	GAGGAGGATCTACAGATTGAGAGCCCGTTTGCAGGGTATACAAATGCTACGAAATGCA	1020	N6	NPSELKRLSSTNIGNSIHEKFEFIFKFEWFEVSSSDMFLERLVDVQTFHNLDELTYEA	120
N7	GAGGAGGATCTACAGATTGAGAGCCCGTTTGCAGGGTATACAAATGCTACGAAATGCA	1020	N7	NPSELKRLSSTNIGNSIHEKFEFIFKFEWFEVSSSDMFLERLVDVQTFHNLDELTYEA	120
Ref	TTGCAGAGCAAACTCAACAGCATTACTGGACCACCTGCAACTGATGACTCTTATGCAATT	1080	Ref	LYERILKITEQLRPTIYWSFPAFLMSHLIFISRAPEYVILNRNCPDNIVLLPIVDLLNH	180
S1	TTGCAGAGCAAACTCAACAGCATTACTGGACCACCTGCAACTGATGACTCTTATGCAATT	1080	S1	LYERILKITEQLRPTIYWSFPAFLMSHLIFISRAPEYVILNRNCPDNIVLLPIVDLLNH	180
S2	TTGCAGAGCAAACTCAACAGCATTACTGGACCACCTGCAACTGATGACTCTTATGCAATT	1080	S2	LYERILKITEQLRPTIYWSFPAFLMSHLIFISRAPEYVILNRNCPDNIVLLPIVDLLNH	180
S3	TTGCAGAGCAAACTCAACAGCATTACTGGACCACCTGCAACTGATGACTCTTATGCAATT	1080	S3	LYERILKITEQLRPTIYWSFPAFLMSHLIFISRAPEYVILNRNCPDNIVLLPIVDLLNH	180
N5	TTGCAGAGCAAACTCAACAGCATTACTGGACCACCTGCAACTGATGACTCTTATGCAATT	1080	N5	LYERILKITEQLRPTIYWSFPAFLMSHLIFISRAPEYVILNRNCPDNIVLLPIVDLLNH	180
N6	TTGCAGAGCAAACTCAACAGCATTACTGGACCACCTGCAACTGATGACTCTTATGCAATT	1080	N6	LYERILKITEQLRPTIYWSFPAFLMSHLIFISRAPEYVILNRNCPDNIVLLPIVDLLNH	180
N7	TTGCAGAGCAAACTCAACAGCATTACTGGACCACCTGCAACTGATGACTCTTATGCAATT	1080	N7	LYERILKITEQLRPTIYWSFPAFLMSHLIFISRAPEYVILNRNCPDNIVLLPIVDLLNH	180
Ref	GATCCTTACAGAGCTATTGTGCTGACGTTTATACTAAAGTCAAAAACAAATTTTAAAA	1140	Ref	DYRSKVKWYPENGFVCEKIGTASQSELSNNGGKGNELLSSGYGFVLEDNIFDSVALK	240
S1	GATCCTTACAGAGCTATTGTGCTGACGTTTATACTAAAGTCAAAAACAAATTTTAAAA	1140	S1	DYRSKVKWYPENGFVCEKIGTASQSELSNNGGKGNELLSSGYGFVLEDNIFDSVALK	240
S2	GATCCTTACAGAGCTATTGTGCTGACGTTTATACTAAAGTCAAAAACAAATTTTAAAA	1140	S2	DYRSKVKWYPENGFVCEKIGTASQSELSNNGGKGNELLSSGYGFVLEDNIFDSVALK	240
S3	GATCCTTACAGAGCTATTGTGCTGACGTTTATACTAAAGTCAAAAACAAATTTTAAAA	1140	S3	DYRSKVKWYPENGFVCEKIGTASQSELSNNGGKGNELLSSGYGFVLEDNIFDSVALK	240
N5	GATCCTTACAGAGCTATTGTGCTGACGTTTATACTAAAGTCAAAAACAAATTTTAAAA	1140	N5	DYRSKVKWYPENGFVCEKIGTASQSELSNNGGKGNELLSSGYGFVLEDNIFDSVALK	240
N6	GATCCTTACAGAGCTATTGTGCTGACGTTTATACTAAAGTCAAAAACAAATTTTAAAA	1140	N6	DYRSKVKWYPENGFVCEKIGTASQSELSNNGGKGNELLSSGYGFVLEDNIFDSVALK	240
N7	GATCCTTACAGAGCTATTGTGCTGACGTTTATACTAAAGTCAAAAACAAATTTTAAAA	1140	N7	DYRSKVKWYPENGFVCEKIGTASQSELSNNGGKGNELLSSGYGFVLEDNIFDSVALK	240
Ref	GAGCGTTTAAACGAGGTTGAAAAAATCAGAAAAACAATGCTGTGACAGAACCAAGCACC	1200	Ref	VKLPDLVSTILETEPFLKPLLSDYTTAFENKDCVQKEKATRSATDVINGVTVFINI	300
S1	GAGCGTTTAAACGAGGTTGAAAAAATCAGAAAAACAATGCTGTGACAGAACCAAGCACC	1200	S1	VKLPDLVSTILETEPFLKPLLSDYTTAFENKDCVQKEKATRSATDVINGVTVFINI	300
S2	GAGCGTTTAAACGAGGTTGAAAAAATCAGAAAAACAATGCTGTGACAGAACCAAGCACC	1200	S2	VKLPDLVSTILETEPFLKPLLSDYTTAFENKDCVQKEKATRSATDVINGVTVFINI	300
S3	GAGCGTTTAAACGAGGTTGAAAAAATCAGAAAAACAATGCTGTGACAGAACCAAGCACC	1200	S3	VKLPDLVSTILETEPFLKPLLSDYTTAFENKDCVQKEKATRSATDVINGVTVFINI	300
N5	GAGCGTTTAAACGAGGTTGAAAAAATCAGAAAAACAATGCTGTGACAGAACCAAGCACC	1200	N5	VKLPDLVSTILETEPFLKPLLSDYTTAFENKDCVQKEKATRSATDVINGVTVFINI	300
N6	GAGCGTTTAAACGAGGTTGAAAAAATCAGAAAAACAATGCTGTGACAGAACCAAGCACC	1200	N6	VKLPDLVSTILETEPFLKPLLSDYTTAFENKDCVQKEKATRSATDVINGVTVFINI	300
N7	GAGCGTTTAAACGAGGTTGAAAAAATCAGAAAAACAATGCTGTGACAGAACCAAGCACC	1200	N7	VKLPDLVSTILETEPFLKPLLSDYTTAFENKDCVQKEKATRSATDVINGVTVFINI	300
Ref	TTGCTAACCATGAGCAAAATTCCTTAAAGAAAGACCCCTGCTTTGACAGAACTGAATACCT	1260	Ref	QNECQLEPFLDLFFYLKSAEEDLDHLRRLQIQMLRNALQSKLNSITGFPATDDSYAI	360
S1	TTGCTAACCATGAGCAAAATTCCTTAAAGAAAGACCCCTGCTTTGACAGAACTGAATACCT	1260	S1	QNECQLEPFLDLFFYLKSAEEDLDHLRRLQIQMLRNALQSKLNSITGFPATDDSYAI	360
S2	TTGCTAACCATGAGCAAAATTCCTTAAAGAAAGACCCCTGCTTTGACAGAACTGAATACCT	1260	S2	QNECQLEPFLDLFFYLKSAEEDLDHLRRLQIQMLRNALQSKLNSITGFPATDDSYAI	360
S3	TTGCTAACCATGAGCAAAATTCCTTAAAGAAAGACCCCTGCTTTGACAGAACTGAATACCT	1260	S3	QNECQLEPFLDLFFYLKSAEEDLDHLRRLQIQMLRNALQSKLNSITGFPATDDSYAI	360
N5	TTGCTAACCATGAGCAAAATTCCTTAAAGAAAGACCCCTGCTTTGACAGAACTGAATACCT	1260	N5	QNECQLEPFLDLFFYLKSAEEDLDHLRRLQIQMLRNALQSKLNSITGFPATDDSYAI	360
N6	TTGCTAACCATGAGCAAAATTCCTTAAAGAAAGACCCCTGCTTTGACAGAACTGAATACCT	1260	N6	QNECQLEPFLDLFFYLKSAEEDLDHLRRLQIQMLRNALQSKLNSITGFPATDDSYAI	360
N7	TTGCTAACCATGAGCAAAATTCCTTAAAGAAAGACCCCTGCTTTGACAGAACTGAATACCT	1260	N7	QNECQLEPFLDLFFYLKSAEEDLDHLRRLQIQMLRNALQSKLNSITGFPATDDSYAI	360
Ref	TCGCTGTCAGCAGCAAGAGTGGTGAAGAGGTCATCTTTGAATCTACTTATGATTTATTG	1320	Ref	DFYRVYCADVYTRGQRIKLEALTRKRLKRTMLSENKRLHMTSKIRLKKDPAFAETELP	420
S1	TCGCTGTCAGCAGCAAGAGTGGTGAAGAGGTCATCTTTGAATCTACTTATGATTTATTG	1320	S1	DFYRVYCADVYTRGQRIKLEALTRKRLKRTMLSENKRLHMTSKIRLKKDPAFAETELP	420
S2	TCGCTGTCAGCAGCAAGAGTGGTGAAGAGGTCATCTTTGAATCTACTTATGATTTATTG	1320	S2	DFYRVYCADVYTRGQRIKLEALTRKRLKRTMLSENKRLHMTSKIRLKKDPAFAETELP	420
S3	TCGCTGTCAGCAGCAAGAGTGGTGAAGAGGTCATCTTTGAATCTACTTATGATTTATTG	1320	S3	DFYRVYCADVYTRGQRIKLEALTRKRLKRTMLSENKRLHMTSKIRLKKDPAFAETELP	420
N5	TCGCTGTCAGCAGCAAGAGTGGTGAAGAGGTCATCTTTGAATCTACTTATGATTTATTG	1320	N5	DFYRVYCADVYTRGQRIKLEALTRKRLKRTMLSENKRLHMTSKIRLKKDPAFAETELP	420
N6	TCGCTGTCAGCAGCAAGAGTGGTGAAGAGGTCATCTTTGAATCTACTTATGATTTATTG	1320	N6	DFYRVYCADVYTRGQRIKLEALTRKRLKRTMLSENKRLHMTSKIRLKKDPAFAETELP	420
N7	TCGCTGTCAGCAGCAAGAGTGGTGAAGAGGTCATCTTTGAATCTACTTATGATTTATTG	1320	N7	DFYRVYCADVYTRGQRIKLEALTRKRLKRTMLSENKRLHMTSKIRLKKDPAFAETELP	420
Ref	SILFSNEDGEEVIFESTYDILLMLILLKTRKNSYPTRYEWVQQVYTFNFGQAVISDDAKAF	440	Ref	SILFSNEDGEEVIFESTYDILLMLILLKTRKNSYPTRYEWVQQVYTFNFGQAVISDDAKAF	440
S1	SILFSNEDGEEVIFESTYDILLMLILLKTRKNSYPTRYEWVQQVYTFNFGQAVISDDAKAF	440	S1	SILFSNEDGEEVIFESTYDILLMLILLKTRKNSYPTRYEWVQQVYTFNFGQAVISDDAKAF	440
S2	SILFSNEDGEEVIFESTYDILLMLILLKTRKNSYPTRYEWVQQVYTFNFGQAVISDDAKAF	440	S2	SILFSNEDGEEVIFESTYDILLMLILLKTRKNSYPTRYEWVQQVYTFNFGQAVISDDAKAF	440
S3	SILFSNEDGEEVIFESTYDILLMLILLKTRKNSYPTRYEWVQQVYTFNFGQAVISDDAKAF	440	S3	SILFSNEDGEEVIFESTYDILLMLILLKTRKNSYPTRYEWVQQVYTFNFGQAVISDDAKAF	440
N5	SILFSNEDGEEVIFESTYDILLMLILLKTRKNSYPTRYEWVQQVYTFNFGQAVISDDAKAF	440	N5	SILFSNEDGEEVIFESTYDILLMLILLKTRKNSYPTRYEWVQQVYTFNFGQAVISDDAKAF	440
N6	SILFSNEDGEEVIFESTYDILLMLILLKTRKNSYPTRYEWVQQVYTFNFGQAVISDDAKAF	440	N6	SILFSNEDGEEVIFESTYDILLMLILLKTRKNSYPTRYEWVQQVYTFNFGQAVISDDAKAF	440
N7	SILFSNEDGEEVIFESTYDILLMLILLKTRKNSYPTRYEWVQQVYTFNFGQAVISDDAKAF	440	N7	SILFSNEDGEEVIFESTYDILLMLILLKTRKNSYPTRYEWVQQVYTFNFGQAVISDDAKAF	440

Supplementary Fig. 1. Multiple sequence alignment of *RKM1* region of NFS and SFS strains of *S. fimicola* with respect to the *Saccharomyces cerevisiae*. Note: Symbol (*) showing conserved sites, space and highlighted regions showing SNPs.

Supplementary Fig. 2. Multiple sequence alignment of amino acid sequence of *RKM1* protein of different strains of *S. fimicola* with respect to the *S. cerevisiae* amino acid sequence to observe the genetic variations. Symbol (*) showing fully conserved sites, symbol (:): depicting conservation between groups of strongly similar properties, space and highlighted regions showing polymorphic sites.

Ref	CTGAACGAAACCGGATCGTGGGAAGGTTAATTATCTGTATACCTTTAGAGATGGAAGTT	60	Ref	AACGCCAATATCAAGAAATCTTGAAGAGTGAAGAAATAGTACTAGATTATATGATGTT	780
N5	CTGAACGAAACCGGATCGTGGGAAGGTTAATTATCTGTATACCTTTAGAGATGGAAGTT	60	N5	AACGCCAATATCAAGAAATCTTGAAGAGTGAAGAAATAGTACTAGATTATATGATGTT	780
N6	CTGAACGAAACCGGATCGTGGGAAGGTTAATTATCTGTATACCTTTAGAGATGGAAGTT	60	N6	AACGCCAATATCAAGAAATCTTGAAGAGTGAAGAAATAGTACTAGATTATATGATGTT	780
N7	CTGAACGAAACCGGATCGTGGGAAGGTTAATTATCTGTATACCTTTAGAGATGGAAGTT	60	N7	AACGCCAATATCAAGAAATCTTGAAGAGTGAAGAAATAGTACTAGATTATATGATGTT	780
S3	CTGAACGAAACCGGATCGTGGGAAGGTTAATTATCTGTATACCTTTAGAGATGGAAGTT	60	S3	AACGCCAATATCAAGAAATCTTGAAGAGTGAAGAAATAGTACTAGATTATATGATGTT	780
S1	CTGAACGAAACCGGATCGTGGGAAGGTTAATTATCTGTATACCTTTAGAGATGGAAGTT	60	S1	AACGCCAATATCAAGAAATCTTGAAGAGTGAAGAAATAGTACTAGATTATATGATGTT	780
S2	CTGAACGAAACCGGATCGTGGGAAGGTTAATTATCTGTATACCTTTAGAGATGGAAGTT	60	S2	AACGCCAATATCAAGAAATCTTGAAGAGTGAAGAAATAGTACTAGATTATATGATGTT	780
Ref	TTGCAAGAAAGAACCGATGGGCGCTTACTTTAAAGTTTGGAAACAAACCAAGCGATATG	120	Ref	TATAATAATGGTGAATTTGGCTCAACTAATACTTTTGGTCCAAATCTTGACAATTTCT	840
N5	TTGCAAGAAAGAACCGATGGGCGCTTACTTTAAAGTTTGGAAACAAACCAAGCGATATG	120	N5	TATAATAATGGTGAATTTGGCTCAACTAATACTTTTGGTCCAAATCTTGACAATTTCT	840
N6	TTGCAAGAAAGAACCGATGGGCGCTTACTTTAAAGTTTGGAAACAAACCAAGCGATATG	120	N6	TATAATAATGGTGAATTTGGCTCAACTAATACTTTTGGTCCAAATCTTGACAATTTCT	840
N7	TTGCAAGAAAGAACCGATGGGCGCTTACTTTAAAGTTTGGAAACAAACCAAGCGATATG	120	N7	TATAATAATGGTGAATTTGGCTCAACTAATACTTTTGGTCCAAATCTTGACAATTTCT	840
S3	TTGCAAGAAAGAACCGATGGGCGCTTACTTTAAAGTTTGGAAACAAACCAAGCGATATG	120	S3	TATAATAATGGTGAATTTGGCTCAACTAATACTTTTGGTCCAAATCTTGACAATTTCT	840
S1	TTGCAAGAAAGAACCGATGGGCGCTTACTTTAAAGTTTGGAAACAAACCAAGCGATATG	120	S1	TATAATAATGGTGAATTTGGCTCAACTAATACTTTTGGTCCAAATCTTGACAATTTCT	840
S2	TTGCAAGAAAGAACCGATGGGCGCTTACTTTAAAGTTTGGAAACAAACCAAGCGATATG	120	S2	TATAATAATGGTGAATTTGGCTCAACTAATACTTTTGGTCCAAATCTTGACAATTTCT	840
Ref	AACGCGTAAATTTTTGGGATGATAAGTAACTGCAACTTTTAAACCCTCACTGTCCCT	180	Ref	TGCCAAATCCAGGTTTATGCAAACTGACACATAAAGCAATGGAAGGCAAGTGGAGGA	900
N5	AACGCGTAAATTTTTGGGATGATAAGTAACTGCAACTTTTAAACCCTCACTGTCCCT	180	N5	TGCCAAATCCAGGTTTATGCAAACTGACACATAAAGCAATGGAAGGCAAGTGGAGGA	900
N6	AACGCGTAAATTTTTGGGATGATAAGTAACTGCAACTTTTAAACCCTCACTGTCCCT	180	N6	TGCCAAATCCAGGTTTATGCAAACTGACACATAAAGCAATGGAAGGCAAGTGGAGGA	900
N7	AACGCGTAAATTTTTGGGATGATAAGTAACTGCAACTTTTAAACCCTCACTGTCCCT	180	N7	TGCCAAATCCAGGTTTATGCAAACTGACACATAAAGCAATGGAAGGCAAGTGGAGGA	900
S3	AACGCGTAAATTTTTGGGATGATAAGTAACTGCAACTTTTAAACCCTCACTGTCCCT	180	S3	TGCCAAATCCAGGTTTATGCAAACTGACACATAAAGCAATGGAAGGCAAGTGGAGGA	900
S1	AACGCGTAAATTTTTGGGATGATAAGTAACTGCAACTTTTAAACCCTCACTGTCCCT	180	S1	TGCCAAATCCAGGTTTATGCAAACTGACACATAAAGCAATGGAAGGCAAGTGGAGGA	900
S2	AACGCGTAAATTTTTGGGATGATAAGTAACTGCAACTTTTAAACCCTCACTGTCCCT	180	S2	TGCCAAATCCAGGTTTATGCAAACTGACACATAAAGCAATGGAAGGCAAGTGGAGGA	900
Ref	GAAGAATAGSAAAAAGGAAGCCAAAGAGATGCATGAAGAATATTAAATCAATCAA	240	Ref	TGCCAAATCCAGGTTTATGCAAACTGACACATAAAGCAATGGAAGGCAAGTGGAGGA	900
N5	GAAGAATAGSAAAAAGGAAGCCAAAGAGATGCATGAAGAATATTAAATCAATCAA	240	N5	TGCCAAATCCAGGTTTATGCAAACTGACACATAAAGCAATGGAAGGCAAGTGGAGGA	900
N6	GAAGAATAGSAAAAAGGAAGCCAAAGAGATGCATGAAGAATATTAAATCAATCAA	240	N6	TGCCAAATCCAGGTTTATGCAAACTGACACATAAAGCAATGGAAGGCAAGTGGAGGA	900
N7	GAAGAATAGSAAAAAGGAAGCCAAAGAGATGCATGAAGAATATTAAATCAATCAA	240	N7	TGCCAAATCCAGGTTTATGCAAACTGACACATAAAGCAATGGAAGGCAAGTGGAGGA	900
S3	GAAGAATAGSAAAAAGGAAGCCAAAGAGATGCATGAAGAATATTAAATCAATCAA	240	S3	TGCCAAATCCAGGTTTATGCAAACTGACACATAAAGCAATGGAAGGCAAGTGGAGGA	900
S1	GAAGAATAGSAAAAAGGAAGCCAAAGAGATGCATGAAGAATATTAAATCAATCAA	240	S1	TGCCAAATCCAGGTTTATGCAAACTGACACATAAAGCAATGGAAGGCAAGTGGAGGA	900
S2	GAAGAATAGSAAAAAGGAAGCCAAAGAGATGCATGAAGAATATTAAATCAATCAA	240	S2	TGCCAAATCCAGGTTTATGCAAACTGACACATAAAGCAATGGAAGGCAAGTGGAGGA	900
Ref	CAAAATGGTGGAGAATTTTACGCTGTAGCGACGCTCTTCCGAGTTTGATAAATTTGCCTAT	300	N6	-----LNETSWEGLIICILYEMEVLCERSRWAPYFKVWNKPSDM	40
N5	CAAAATGGTGGAGAATTTTACGCTGTAGCGACGCTCTTCCGAGTTTGATAAATTTGCCTAT	300	N7	-----LNETSWEGLIICILYEMEVLCERSRWAPYFKVWNKPSDM	40
N6	CAAAATGGTGGAGAATTTTACGCTGTAGCGACGCTCTTCCGAGTTTGATAAATTTGCCTAT	300	N5	-----LNETSWEGLIICILYEMEVLCERSRWAPYFKVWNKPSDM	40
N7	CAAAATGGTGGAGAATTTTACGCTGTAGCGACGCTCTTCCGAGTTTGATAAATTTGCCTAT	300	S2	-----LNETSWEGLIICILYEMEVLCERSRWAPYFKVWNKPSDM	40
S3	CAAAATGGTGGAGAATTTTACGCTGTAGCGACGCTCTTCCGAGTTTGATAAATTTGCCTAT	300	S3	-----LNETSWEGLIICILYEMEVLCERSRWAPYFKVWNKPSDM	40
S1	CAAAATGGTGGAGAATTTTACGCTGTAGCGACGCTCTTCCGAGTTTGATAAATTTGCCTAT	300	S1	-----LNETSWEGLIICILYEMEVLCERSRWAPYFKVWNKPSDM	40
S2	CAAAATGGTGGAGAATTTTACGCTGTAGCGACGCTCTTCCGAGTTTGATAAATTTGCCTAT	300	S2	-----LNETSWEGLIICILYEMEVLCERSRWAPYFKVWNKPSDM	40
Ref	ATTGCAAGCATTATATTAGTACTCTTTGATTTGGAAATGCAAGATAGTAGTGTAAAT	360	N6	NALIFWDDNELQLLPSFLVERIGKKEAKEMHERIIRSIKIQIGGFPSRVATSFEDNFAY	100
N5	ATTGCAAGCATTATATTAGTACTCTTTGATTTGGAAATGCAAGATAGTAGTGTAAAT	360	N7	NALIFWDDNELQLLPSFLVERIGKKEAKEMHERIIRSIKIQIGGFPSRVATSFEDNFAY	100
N6	ATTGCAAGCATTATATTAGTACTCTTTGATTTGGAAATGCAAGATAGTAGTGTAAAT	360	S2	NALIFWDDNELQLLPSFLVERIGKKEAKEMHERIIRSIKIQIGGFPSRVATSFEDNFAY	100
N7	ATTGCAAGCATTATATTAGTACTCTTTGATTTGGAAATGCAAGATAGTAGTGTAAAT	360	S3	NALIFWDDNELQLLPSFLVERIGKKEAKEMHERIIRSIKIQIGGFPSRVATSFEDNFAY	100
S3	ATTGCAAGCATTATATTAGTACTCTTTGATTTGGAAATGCAAGATAGTAGTGTAAAT	360	S1	NALIFWDDNELQLLPSFLVERIGKKEAKEMHERIIRSIKIQIGGFPSRVATSFEDNFAY	100
S1	ATTGCAAGCATTATATTAGTACTCTTTGATTTGGAAATGCAAGATAGTAGTGTAAAT	360	S2	NALIFWDDNELQLLPSFLVERIGKKEAKEMHERIIRSIKIQIGGFPSRVATSFEDNFAY	100
S2	ATTGCAAGCATTATATTAGTACTCTTTGATTTGGAAATGCAAGATAGTAGTGTAAAT	360	S3	NALIFWDDNELQLLPSFLVERIGKKEAKEMHERIIRSIKIQIGGFPSRVATSFEDNFAY	100
Ref	GAAGAATAGSAAAAAGGAAGCCAAAGAGATGCATGAAGAATATTAAATCAATCAA	420	N6	IASIILSYSFLEMQDSSVNEEEEESEELENERVLSKSMIFLADLNADTSKCNANLT	160
N5	GAAGAATAGSAAAAAGGAAGCCAAAGAGATGCATGAAGAATATTAAATCAATCAA	420	N7	IASIILSYSFLEMQDSSVNEEEEESEELENERVLSKSMIFLADLNADTSKCNANLT	160
N6	GAAGAATAGSAAAAAGGAAGCCAAAGAGATGCATGAAGAATATTAAATCAATCAA	420	N5	IASIILSYSFLEMQDSSVNEEEEESEELENERVLSKSMIFLADLNADTSKCNANLT	160
N7	GAAGAATAGSAAAAAGGAAGCCAAAGAGATGCATGAAGAATATTAAATCAATCAA	420	S2	IASIILSYSFLEMQDSSVNEEEEESEELENERVLSKSMIFLADLNADTSKCNANLT	160
S3	GAAGAATAGSAAAAAGGAAGCCAAAGAGATGCATGAAGAATATTAAATCAATCAA	420	S3	IASIILSYSFLEMQDSSVNEEEEESEELENERVLSKSMIFLADLNADTSKCNANLT	160
S1	GAAGAATAGSAAAAAGGAAGCCAAAGAGATGCATGAAGAATATTAAATCAATCAA	420	Ref	IASIILSYSFLEMQDSSVNEEEEESEELENERVLSKSMIFLADLNADTSKCNANLT	160
S2	GAAGAATAGSAAAAAGGAAGCCAAAGAGATGCATGAAGAATATTAAATCAATCAA	420	S1	IASIILSYSFLEMQDSSVNEEEEESEELENERVLSKSMIFLADLNADTSKCNANLT	160
Ref	ATGATCCCACTTGCCTGATATGTTGAATGCAAGATACCAAGTAAATGCAACCGTAAATTAAC	480	N6	YDNSCLMVALRDIEKNEQVYNIYGEHNSLLRRYGVVWDGSKYDFGVEVLENIIVEA*	219
N5	ATGATCCCACTTGCCTGATATGTTGAATGCAAGATACCAAGTAAATGCAACCGTAAATTAAC	480	N7	YDNSCLMVALRDIEKNEQVYNIYGEHNSLLRRYGVVWDGSKYDFGVEVLENIIVEA*	219
N6	ATGATCCCACTTGCCTGATATGTTGAATGCAAGATACCAAGTAAATGCAACCGTAAATTAAC	480	N5	YDNSCLMVALRDIEKNEQVYNIYGEHNSLLRRYGVVWDGSKYDFGVEVLENIIVEA*	219
N7	ATGATCCCACTTGCCTGATATGTTGAATGCAAGATACCAAGTAAATGCAACCGTAAATTAAC	480	S3	YDNSCLMVALRDIEKNEQVYNIYGEHNSLLRRYGVVWDGSKYDFGVEVLENIIVEA*	220
S3	ATGATCCCACTTGCCTGATATGTTGAATGCAAGATACCAAGTAAATGCAACCGTAAATTAAC	480	Ref	YDNSCLMVALRDIEKNEQVYNIYGEHNSLLRRYGVVWDGSKYDFGVEVLENIIVEA*	220
S1	ATGATCCCACTTGCCTGATATGTTGAATGCAAGATACCAAGTAAATGCAACCGTAAATTAAC	480	N6	KETFETNFEFLDRCIDILRNANIQFLEGESEIVLSDYDCYNNSELLPQLILLVQILITL	279
S2	ATGATCCCACTTGCCTGATATGTTGAATGCAAGATACCAAGTAAATGCAACCGTAAATTAAC	480	N7	KETFETNFEFLDRCIDILRNANIQFLEGESEIVLSDYDCYNNSELLPQLILLVQILITL	279
Ref	TACGACTCTAACTGTTTAAAGATGGTGTCTTTGAGGATATTAAAAGAAATGAACAAGTG	540	N5	KETFETNFEFLDRCIDILRNANIQFLEGESEIVLSDYDCYNNSELLPQLILLVQILITL	279
N5	TACGACTCTAACTGTTTAAAGATGGTGTCTTTGAGGATATTAAAAGAAATGAACAAGTG	540	S2	KETFETNFEFLDRCIDILRNANIQFLEGESEIVLSDYDCYNNSELLPQLILLVQILITL	280
N6	TACGACTCTAACTGTTTAAAGATGGTGTCTTTGAGGATATTAAAAGAAATGAACAAGTG	540	S3	KETFETNFEFLDRCIDILRNANIQFLEGESEIVLSDYDCYNNSELLPQLILLVQILITL	280
N7	TACGACTCTAACTGTTTAAAGATGGTGTCTTTGAGGATATTAAAAGAAATGAACAAGTG	540	Ref	KETFETNFEFLDRCIDILRNANIQFLEGESEIVLSDYDCYNNSELLPQLILLVQILITL	280
S3	TACGACTCTAACTGTTTAAAGATGGTGTCTTTGAGGATATTAAAAGAAATGAACAAGTG	540	N6	-----	299
S1	TACGACTCTAACTGTTTAAAGATGGTGTCTTTGAGGATATTAAAAGAAATGAACAAGTG	540	N7	-----	299
S2	TACGACTCTAACTGTTTAAAGATGGTGTCTTTGAGGATATTAAAAGAAATGAACAAGTG	540	S2	-----	300
Ref	TACAAACATATACGGAGAACATCCAAATTCGGAACACTAAGAAGATATGGGTATGTTGAA	600	S3	-----	299
N5	TACAAACATATACGGAGAACATCCAAATTCGGAACACTAAGAAGATATGGGTATGTTGAA	600	Ref	-----	300
N6	TACAAACATATACGGAGAACATCCAAATTCGGAACACTAAGAAGATATGGGTATGTTGAA	600	S1	-----	280
N7	TACAAACATATACGGAGAACATCCAAATTCGGAACACTAAGAAGATATGGGTATGTTGAA	600	N6	-----	299
S3	TACAAACATATACGGAGAACATCCAAATTCGGAACACTAAGAAGATATGGGTATGTTGAA	600	N7	-----	299
S1	TACAAACATATACGGAGAACATCCAAATTCGGAACACTAAGAAGATATGGGTATGTTGAA	600	S2	-----	300
S2	TACAAACATATACGGAGAACATCCAAATTCGGAACACTAAGAAGATATGGGTATGTTGAA	600	S3	-----	299
Ref	TGGAGCGTTTCAAGATGATTTTGGAGAAAGTGTACTTGAATAATTTGCGAGGCGTTA	660	Ref	-----	300
N5	TGGAGCGTTTCAAGATGATTTTGGAGAAAGTGTACTTGAATAATTTGCGAGGCGTTA	660	S1	-----	300
N6	TGGAGCGTTTCAAGATGATTTTGGAGAAAGTGTACTTGAATAATTTGCGAGGCGTTA	660	Ref	-----	300
N7	TGGAGCGTTTCAAGATGATTTTGGAGAAAGTGTACTTGAATAATTTGCGAGGCGTTA	660	S1	-----	300
S3	TGGAGCGTTTCAAGATGATTTTGGAGAAAGTGTACTTGAATAATTTGCGAGGCGTTA	660	Ref	-----	300
S1	TGGAGCGTTTCAAGATGATTTTGGAGAAAGTGTACTTGAATAATTTGCGAGGCGTTA	660	S1	-----	300
S2	TGGAGCGTTTCAAGATGATTTTGGAGAAAGTGTACTTGAATAATTTGCGAGGCGTTA	660	Ref	-----	300
Ref	AAAGAGACTTTTGGAGCGAATGATGATTTTGGACAGGTGATGATATCTTACGCCAAT	720	Ref	-----	300
N5	AAAGAGACTTTTGGAGCGAATGATGATTTTGGACAGGTGATGATATCTTACGCCAAT	720	N6	-----	299
N6	AAAGAGACTTTTGGAGCGAATGATGATTTTGGACAGGTGATGATATCTTACGCCAAT	720	N7	-----	299
N7	AAAGAGACTTTTGGAGCGAATGATGATTTTGGACAGGTGATGATATCTTACGCCAAT	720	S2	-----	300
S3	AAAGAGACTTTTGGAGCGAATGATGATTTTGGACAGGTGATGATATCTTACGCCAAT	720	S3	-----	299
S1	AAAGAGACTTTTGGAGCGAATGATGATTTTGGACAGGTGATGATATCTTACGCCAAT	720	Ref	-----	300
S2	AAAGAGACTTTTGGAGCGAATGATGATTTTGGACAGGTGATGATATCTTACGCCAAT	720	S1	-----	300

Supplementary Fig. 3. Multiple sequence alignment of *RKM4* region of *S. fimicola* strains with respect to *S. cerevisiae* in order to observe single nucleotide polymorphism (SNP). Note: Symbol (*) is showing fully conserved sites, space and highlighted regions showing SNPs.

Supplementary Fig. 4. Multiple sequence alignment of amino acid sequence of *RKM4* protein of different strains of *S. fimicola* with respect to the reference strain *S. cerevisiae* amino acid sequence to spot genetic diversity. Symbol (*) showing conserved sites, space and highlighted regions showing polymorphic sites.