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Marker Assisted Screening of *Oryza Rufipogon* accessions for sheath blight tolerance

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Abstract
Potential resistant source against sheath blight of rice, caused by the fungus *Rhizoctonia solani* Kuhn, need to be identified from the wild rice species since no resistant source is reported till now from the cultivated rice germplasm. Eighteen *Oryza rufipogon* accessions along with tolerant check CR 1014 and susceptible check Swarna were phenotyped for sheath blight tolerance for three consecutive seasons. The accessions were also genotyped using 21 biotic stress responsive cgSSR markers which target the sequence variations in genes which are non-specifically expressed under various biotic stress situations in rice. Two wild rice accessions (AC100444 and AC 100015) were found to be moderately resistant to sheath blight. The cgSSR markers were amplified in both the species indicating their cross transferability across species. Though gene sequence based, the markers were highly polymorphic with the PIC value ranging from 0 to 0.85 with an average value of 0.55. The UPGMA clustering based on 106 marker alleles of 21 cgSSR loci grouped the twenty genotypes into three sub-groups. The first sub- group consisted mainly of tolerant accessions; the second sub- group had predominantly susceptible ones and geographically distinct accessions formed a third subgroup irrespective of their sheath blight phenotype. The two wild rice accessions which are found to be moderately resistant to sheath blight and the completely cross transferable and highly polymorphic cgSSR markers reported in the study can be utilized in future biotic stress breeding programmes.

Keywords cgSSR, Genotyping, Sheath Blight, Wild Rice

Introduction
Rice is the most important food cereal of the world. More than half of the world population relies on rice for deriving dietary nutrition and energy (Yu *et al*. 2002). Hence any loss, whether pre-harvest or post-harvest, caused by biotic or abiotic stress cannot be afforded given the increasing requirement of it for the ever increasing human population. But, biotic stresses, including diseases and pests cause severe yield reduction in rice. The Bengal famine of 1943 caused due to the brown spot disease of rice, where more than 2 million people lost their lives, is an ideal example of what a single disease is capable of causing to the welfare of the human population. Sheath blight of rice, caused by the fungus, *Rhizoctonia solani* Kuhn, is becoming a major threat to rice production worldwide (Savary *et al*. 2006). Though first reported as early as in 1910, sheath blight became a prominent disease only after the introduction of high yielding semi dwarf varieties in the 1960's. The intensive cropping involving cultivation of a single variety over a large area and the high use of nitrogenous fertilizer led to a dramatic increase in the incidence of sheath blight in major rice growing countries of the world including China (Chen *et al*., 2012), Japan (JPPA, 2013) and USA (Prasad and Eizenga, 2008). With the current emphasis on intensive cropping aimed at producing more crop per unit area, the disease is predicted to become more severe in the coming days, and may become the most important biotic stress affecting rice production worldwide. Though the pathogen can be controlled by spraying fungicides, its impact on the environment, on the quality of the produce and also the economic aspects compels us to go for a more sustainable and eco-friendly way of taming the disease (Jia *et al.,* 2012). Development of genotypes tolerant to the disease is considered as the most sustainable, eco-friendly and economic way to combat the disease (Hossain *et al*., 2016).
Breeding of tolerant genotypes for sheath blight tolerance in rice poses many unique challenges compared to other pests and diseases. Potential resistant source against sheath blight of rice need to be identified from the wild rice species since no resistant source is reported till now from the cultivated rice germplasm (Channamallikarjuna *et al*., 2010; Yadav *et al*., 2015). Considering the fact that there is 50-60% reduction in the number of alleles in cultivated rice compared to wild rice (Sun *et al*., 2001), there is a need to screen and identify

Sheath blight resistant sources from the wild rice species. Many useful traits, especially, tolerance to various biotic and abiotic stresses, were already introgressed into the cultivated rice from the wild species. Among the wild relatives of rice, the diploid species *Oryza rufipogon* (2n=2x=24) has special importance in rice breeding programmes. Being in the primary gene pool of rice, the species yield fertile hybrids when crossed with rice, hence the traits of interest can be easily introgressed (Haritha *et al.*, 2017).

But screening and identification of true resistant lines against sheath blight is very difficult even in cultivated rice and all the more difficult in case of wild rice species because of their spreading habit (Eizenga *et al*., 2002). A well standardized method for assessing the disease severity is not yet finalized for sheath blight (Park *et al.*, 2008). Moreover, sheath blight tolerance in rice is a quantitative trait governed by polygenes (Pinson *et al*. 2005). The trait is expressed in constant interaction with the environmental conditions. Disease development and expression is so significantly influenced by the prevailing environment that, screening and selection of truly resistant lines becomes a very difficult task (Jia *et al*., 2007; Park *et al.*, 2008). Hence, to establish the true resistance of an accession and to utilize it in breeding programmes, screening needs to be repeated multiple times over different locations and environmental conditions. Candidate gene based SSR markers (cgSSR) can be of immense utility in germplasm evaluation experiments. Molla *et al.* (2016) reported that cgSSR markers can very clearly classify salinity tolerant and susceptible rice genotypes into two distinguishing panels, thus complementing the phenotypic evaluation of the trait in a germplasm. But the utility of such cgSSR markers have not been tested for germplasm evaluation of a quantitative trait like sheath blight tolerance.

Hence the present study was formulated to identify potential resistant sources to sheath blight in the wild rice (*Oryza rufipogon*) germplasm. The efficacy cgSSR markers in differentiating germplasm accessions having contrasting phenotypes for sheath blight tolerance was studied and the concept of a more reliable and robust molecular marker assisted screening for sheath blight tolerance was tested.

Materials and methods

Plant and Fungal Material

18 *Oryza rufipogon* accessions and two rice varieties, CR 1014 and Swarna, were screened for sheath blight tolerance under net house conditions for three successive rainy seasons (2014-2016). The genotypes were screened in the summer season also, but the disease development was poor hence the data are not considered. The variety CR 1014 was released from the National Rice Research Institute, Cuttack, India, and is consistently showing moderately resistant reaction to sheath blight, hence used as the tolerant check. The variety Swarna, is the most predominant rice variety in the Indian subcontinent, but it is highly susceptible to sheath blight, hence used as the susceptible check. The plants were raised in earthern pots in three replications with more than three plants in each replication. A minimum of three plants in each pot were inoculated with the sheath blight isolate, ShbSl4 of the fungus *R solani.* The inoculation was done by inserting the sclerotia with mycelia of the fungus, inside the leaf sheaths during the maximum tillering stage of the plants. Proper humidity was maintained throughout the screening period in order for the proper disease development. The data on sheath blight reactions of the accessions were recorded 3 times in 10 days interval. The disease severity was assessed in a scale of 0 to 9 (SES, 1996); with 0 being the most resistant and 9 being the highly susceptible. Accessions with scores of 0-1 were classified as resistant, 1-3 as moderately resistant, 3-5 as tolerant, 5-7 as susceptible and 7-9 as highly susceptible. The average disease scores for each accession for the three seasons were calculated and used for correlating with the molecular screening data.

Primer Designing

An extensive literature survey was carried out to identify biotic stress responsive candidate genes in rice. This revealed that there are 296 candidate genes which respond to various biotic stresses including insect and pathogen infection. Among the disease responsive genes, some are expressed specifically against a particular pathogen, e.g., *pi* genes against the blast pathogen and *Xa* genes against the bacterial leaf blight pathogen. But there are genes which are expressed non-specifically under different biotic stress situations. Such genes are ideal candidates for the screening of a germplasm for sheath blight tolerance. Sequence variations in those genes are supposed to have an effect on the phenotypic response to various biotic stresses including sheath blight. Genes which are expressed at different levels of the defense response cascade were selected and the sequences were analyzed for the presence of microsatellite repeats using the SSR IT tool (Temnykh *et al*., 2001). Considering the length of the microsatellite repeats and the chances of getting polymorphism, 21 primers were designed to target the length variations present in those repeats. The 20 accessions were genotyped using these primers designed.

Genotyping of the Screening Materials

The DNA of all the 20 genotypes was isolated using CTAB method proposed by Doyle and Doyle (1990). For doing the PCR reaction, DNA and cgSSR primers were diluted to get working solutions of 20 ng/µl and 5 pmole, respectively. PCR reactions were performed in a 10µl final volume containing 20 ng of genomic DNA and the GoTaq Green Master Mix (Promega). The reaction conditions were as follows: 94^{o}C for 4 minutes, 94^{o}C for 1 minute, 55^{o}C for 1 minute, 72^{o}C for 2 minutes, final extension at 72^{o}C for 7 minutes, and final cooling at 4^{o}C. The reactions were set for 40 cycles. The amplified PCR products were subsequently resolved on 3.5% metaphore gel in 1X TBE buffer.

Data Analysis

Each amplified band was considered as an allele and the molecular weight for the sample bands were calculated based on standards (50bp ladder, Thermo scientific, USA) from both the lanes. The band analysis was done using the Alpha View software version 3.4.0 (Protein Simple, USA). If a particular allele is present for a locus in an accession, it is denoted as 1 and the absence was denoted as zero. The resultant data matrix was used for further analyses. An allele is considered as unique when it is present in only one accession and alleles are designated as rare when they are present in less than 5% of the accessions. Expected heterozygosity (H) was calculated as a measure of gene diversity based on the formula proposed by Nei (1973) and Brown and Weir (1983). The relative value of each primer with respect to the amount of polymorphism exhibited is estimated as the polymorphic information content (PIC) value, as per Botstein *et al.* (1980)**.** The dissimilarity matrix prepared based on the presence or absence of a particular allele in a particular accession was used as the input for the metric multidimensional scaling or principal

coordinate analysis (PCoA). The factorial analysis on dissimilarity of the accessions was carried out using DARwin v 6.0.14. The phylogentic relationships among the accessions were plotted as a dendrogram employing the un-weighted hierarchical clustering method.

Results and discussion

Screening for Sheath Blight Tolerance

Sheath blight is becoming the most important biotic stress affecting rice production worldwide. The prevalence of the disease is predicted to increase in the future when the agriculture become more intensive aiming maximum production per unit area. Though development of tolerant cultivars is considered as the best strategy to combat the disease, identification of potential donor lines has been an obstacle to achieve this. Screening of a wide range of cultivated rice accessions in the past yielded few cultivars, *viz*., Teqing, Tetep, Jasmine 85 etc (Channamallikarjuna *et al*., 2010; Yadav *et al*., 2015), showing moderately resistant phenotype for sheath blight. But, unlike the case with many of the diseases and insect pests, a true resistant source against sheath blight could not be identified in the cultivated rice germplasm (Jia *et al*., 2007; Liu *et al.,* 2009). Hence, it is necessary to explore the wild rice germplasm resources for identifying potential donors for sheath blight tolerance. Prasad and Eizenga (2008) reported moderate resistance against sheath blight in accessions of *Oryza* species like *O. nivara, O. barthii, O. meridionalis and O. officinalis*. But such reports in accessions of *O. rufipogon* are very limited. In the present study involving screening of eighteen *O. rufipogon* accessions and two cultivated rice cultivars, there were significant differences in disease reaction for sheath blight tolerance among the accessions (*F* value: 11.76, $p<0.00$). But, no accession showed a resistant reaction (<1 disease score) for sheath blight based on the average disease score of the screening experiment for three seasons (Table 1). Apart from the tolerant check CR 1014, two wild rice accessions, AC 100444 and AC 100015, showed average disease scores of 2.30 and 2.70, respectively, hence classified as moderately resistant to sheath blight. These two accessions showed consistent phenotype for sheath blight tolerance for all the three seasons studied. Hence these can be used for haplotype analysis to determine whether they carry the same sheath blight resistant QTLs reported in previous studies. If they carry novel resistance QTLs for sheath blight tolerance, they can be further utilized for mapping and as donor lines in marker assisted introgression of resistance QTLs into otherwise superior but sheath blight susceptible genotypes. Four accessions (AC 100005, AC 100263, AC 100380 and AC 100493) showed disease scores in the range of 3 to 5, hence classified as tolerant whereas 12 accessions were found to be susceptible with a disease score of more than 5. Only the susceptible check, Swarna, showed high susceptibility to the disease with an average disease score of 7.20.

The inability to identify a genotype with absolute immune reaction to sheath blight till date can be attributed to the genetic control of the trait. Previous studies unambiguously established that sheath blight tolerance in rice is a polygenic trait controlled by many genes (Pinson *et al*., 2005). Hence the ideal method to combat the disease will be to identify resistance QTLs from various genotypes and introgress them into superior but sheath blight susceptible rice varieties.

Genotyping of Accessions Using Designed Primers

Specific resistance genes of major biotic stresses like blast, bacterial leaf blight, gall midge and brown plant hopper have been identified in rice. Gene sequence based molecular markers have been developed for these traits which are of immense importance in the screening of germplasm resources for differentiating genotypes showing contrasting phenotypes and also in the marker assisted transfer of the traits to desirable genetic background. But there is no single report of identification of a major resistance gene for sheath blight tolerance in rice (Jia *et al*., 2009; Zuo *et al*., 2010; Liu *et al*., 2013). This makes the marker assisted screening for sheath blight tolerance difficult. The inability to identify a major resistance gene against sheath blight of rice might be attributed to the necrotrophic nature of the pathogen. The host plant response, when subjected to a biotic stress by a necrotrophic pathogen, is not the result of the expression of a single or few genes (Zhao *et al*., 2008). The stress response is a very complex trait involving an altered expression of several genes at different levels of the cascade comprising recognition, signaling, transcription and the final defense response. Molecular markers can be developed to target the length variations in the sequences of these genes and the same can be correlated with an accessions' phenotype for necrotrophic diseases like sheath blight.

Among the many types of molecular markers developed, the markers based on the variation in simple sequence repeats (SSRs) in the genome were the most widely used. SSR markers are co-dominant; PCR based, targets a single locus, and can have more alleles than any other molecular marker. But SSR markers are primarily designed to detect neutral variations present in the non-genic sequence of the genome. The probabilities of association of such variations with the phenotype are very less. Since the non-genic regions are less conserved during evolution, the cross transferability of such markers are also limited. Hence, in a study involving both cultivated and wild species, markers which are based on the highly conserved genic regions of the genome need to be used. Such gene sequence based markers are more cross transferable across different species and their polymorphisms will have a better association with the phenotype. In the present study an extensive literature survey was carried out to identify biotic stress responsive candidate genes in rice. Among the 296 genes identified, genes which are expressed at different levels of the disease response cascade during infection by necrotrophic pathogens were selected. The sequences of these genes were analyzed for the presence of microsatellite repeats. Based on the length of microsatellite repeats in the sequence, 21 genes were shortlisted for primer synthesis. OsWRKY4, which is a candidate gene for sheath blight tolerance in rice (Wang *et al*., 2015) and has a tri-nucleotide repeat (TTC) in the CDS region, was also included. The gene sequence based SSR markers designed for these twenty one genes are listed in Table 2. The twenty genotypes which were phenotyped for sheath blight tolerance were screened using these primers. All the twenty one primers were amplified in both *Oryza sativa* and *Oryza rufipogon* species indicating the cross transferability of the designed markers. A representative gel picture showing banding pattern of lipoxygenase gene (LOC_Os03g49380) is shown in Fig.1. Except two markers, OsWRKY76 (LOC_Os09g25060) and OsPR10 (LOC_Os03g18850), all others were polymorphic among the accessions genotyped. A total of 106 alleles were detected for the twenty one primers used, with an average of 5 alleles per locus. The number of alleles per locus varied from one for the two monomorphic markers to ten for the marker, OsAOC (LOC_Os03g32314). Though gene sequence based

markers are used in the study, which are supposed to be highly conserved across species, a very high level of gene diversity was observed, with the expected heterozygosity (He) value ranging from 0 to 0.85 and an average He value of 0.59. This is mainly due to the presence of heterozygotic loci in the *Oryza rufipogon* accessions, which indicates presence of cross pollination in the wild rice species. The markers were highly polymorphic among the accessions with the PIC value ranging from 0 to 0.85 with an average value of 0.554, indicating the usefulness of the markers used in differentiating germplasm resources with varying degree of sheath blight tolerance.

The genotyping data was used to plot the dendrogram depicting the evolutionary relationships among the accessions studied (Fig. 2). The UPGMA clustering based on 106 marker alleles of 21 cgSSR loci grouped the twenty genotypes used in the study into three sub-groups. The first sub group consists mainly of moderately resistant and tolerant accessions, the second sub group consists mainly the susceptible accessions and three accessions collected from a particular geographical area forming the third sub-group. The most tolerant of all the accessions screened for sheath blight tolerance, the two moderately resistant wild rice accessions, AC 100444 (SES score, 2.30) and AC 100015 (SES score, 2.70) and one tolerant accession, AC 100005 (SES score, 3.40) were grouped in the first sub-group along with the tolerant check CR 1014 (SES score, 2.70). Though exhibiting contrasting phenotypes for sheath blight tolerance, the two *Oryza sativa* cultivars, Swarna and CR 1014 were grouped in the same sub-sub-group indicating their evolutionary relationships in comparison to *Oryza rufipogon* accessions. Four susceptible accessions, AC 100047 (SES score, 6.10), AC 100166 (SES score, 5.20), AC 100168 (SES score, 5.30) and AC 100174 (SES score, 6.00) were also included in the same sub-group. The second sub-group consists mainly of susceptible accessions; out of the total eight accessions included in the sub-group, six are susceptible and two are tolerant. The wild rice accessions collected from the Indian state of Tripura, the sheath blight susceptible AC 100373 (SES score, 6.00) and AC 100492 (SES score, 5.10) and the sheath blight tolerant AC 100380 (SES score, 4.60) were grouped together in the third sub-group. There are reports that candidate gene based SSR markers can completely differentiate germplasm resources having contrasting phenotypes into two panels, e.g., salinity tolerant and susceptible cultivars (Molla *et al*., 2016). In contrast to such reports, the cgSSR markers used in the present study could not differentiate germplasm resources showing varying sheath blight tolerance. Though there is a pattern in the grouping of tolerant and susceptible genotypes into different sub-groups, both susceptible and tolerant genotypes featured in all the three sub-groups. This can be attributed mainly to the fact that sheath blight tolerance in rice is a quantitative trait governed by polygenes, and that even a susceptible variety can contribute resistant alleles as reported in previous studies (Zuo *et al*., 2011) where the tolerant allele of the qSB-11^{LE} QTL was contributed by the susceptible genotype Lemont. Hence it can be concluded from the results obtained from the current study that cgSSR markers may give preliminary information on the phenotypic nature of a quantitative trait and can be used to complement the conventional germplasm evaluation experiments for such traits.

The two wild rice accessions consistently showing moderately resistant reaction to sheath blight can be utilized in breeding programmes aimed at development of sheath blight tolerant rice varieties. The completely cross transferable and highly polymorphic cgSSR markers of important defense related genes reported in the study can be used in future stress breeding programmes, especially those involving a wild species. The markers showing polymorphism between wild and cultivated rice species can be used in the marker assisted introgression of biotic stress tolerance in rice.

Table 1: Screening of *Oryza rufipogon* accessions for three seasons with *R solani* AG1-1A/ ShbSl4

Genotype	Disease score (Mean±S.E.)	Disease reaction	DMRT (5%)
Swarna	7.20±0.321	HS	a
AC 100173	6.70±0.351	S	ab
AC 100014	6.70±0.513	S	ab
AC 100047	6.10±0.404	S	abc
AC 100373	6.00±0.586	S	abcd
AC 100174	6.00±0.416	S	abcd
AC 100102	5.70±0.379	S	bcd
AC 100495	5.60±0.400	S	bcd
AC 100494	5.30±0.493	S	bcd
AC 100168	5.30±0.321	S	bcd
AC 100019	5.20±0.451	S	cde
AC 100166	5.20±0.503	S	cde
AC 100492	5.10±0.416	S	cde
AC 100380	4.60±0.656	T	def
AC 100493	3.80±0.361	T	fg
AC 100005	3.40±0.458	T	fgh
AC 100263	3.10±0.265	T	gh
AC 100015	2.70±0.321	MR	gh
CR 1014	2.70±0.361	MR	gh
AC 100444	2.30±0.321	MR	h

HS: highly susceptible; S: susceptible; MS: moderately susceptible; MR: moderately resistant; R: resistant; HR: highly resistant

Table 2. Details of cgSSR primers used for genotyping accessions showing varying degree of sheath blight tolerance

Gene	Locus ID	SSR motif	Position	Primer	Band size (bp)	No. of alleles	PIC value	He value
OsBRR1	Os03g12730.1	(CTC)9	CDS	F: 5' CTAACAACCGAAACAGAAAGAACACG R: 5' CACGAGCAGAACGCGGTGTC	315	4	0.47	0.56
OsGAP1	Os02g22130	(TC)10	5' UTR	F: 5' GGTCCAGAGGTTTATCGTGTTCAGG R:5'CAAGCAGCAAAAGGTACACAATACATG	186	3	0.45	0.51
OsRacB	Os02g02840.1	(GA)21	5'UTR	F:5'ATCTGCGAGAAACCCTCTCCG R:5'CGACGGTGACGCACTTTATGA	270	6	0.71	0.75
OsRacB	Os02g02840.1	(TTC)9	Intron	F:5'GCGCGTCCAGGTTCATAAAGT R:5'CAAGCCTCCGAGCACACAAGT	224	6	0.75	0.78
OsWRKY4	Os03g55164.1	(TTC)13	CDS	F:5'CAAGTGGAATGGAGCATTCTAC R:5'GAATTGCTGACGAGAAGAACACAAGC	167	6	0.79	0.82
OsWRKY13	Os01g54600.1	(GA)15	5' UTR	F:5'GCCATGCGTACATACACGTTCATG R:5'ATGGGTGCAGCTTTCAATGATCTC	246	7	0.80	0.83
OsWRKY76	Os09g25060.1	(TTGA)9	Intron	F:5'AGAGAGACGCGAGCTGTTCGTG R:5'CAAAACCAAGCAAGAACCGAATCC	292	1	0.00	0.00
OsWRKY82	Os08g17400	(TA)12	Intron	F:5'AGCTTAGTTGATGGTAACTGTGGGAA R:5'CACACAGCATTATATTAGGCAGTATGATG	189	4	0.39	0.42
OsWRKY 83	Os12g40570	(GGC)9	CDS	F:5'AGAATCCGAGGCTTCTCTTGCTG R:5'CATCCTGCCTCTAGCATGTCCAC	274	9	0.74	0.77
OsSWN2	Os08g02300.1	(AG)10	3' UTR	F:5'TGCCACCCTAGCTGCTAGTACAGT R:5'CACTCGATCTTTGCTAATCACCTTTG	178	4	0.44	0.48
Gns1/OsEGL1	Os05g31140	(TC)11	Intron	F:5'GGACTATCCAACGGTCATGCTG R:5'CGGAGATGGACAAGGAATATACTACC	151	8	0.81	0.83
OsPR1a	Os07g03710	(TA)9	5' UTR	F:5'TCGATCTCCATCATCTCTTCGTC Reverse 5' AAGTCCTGCGCCGAGTTCT	240	7	0.59	0.66
JIOsPR10	Os03g18850	(TA)9	Intron	F:5'CGTCAGGCAGTTCAACTTCACCTC R:5'AACGTGTTGGGGATTGAACACG	161	1	0.00	0.00
OsNPR3/NH3	Os03g46440	(GT)9	5' UTR,	F:5'GTTCTTGTGTCGTGTGGTGGGG Reverse 5'CAAACCCAAGAACGGGAAATTCTC	177	4	0.53	0.57
OsPR1#052	Os05g51680	(TTTA)9	Intron	F:5'CATTCCATTCCTCCACCCACAC R:5'CCTTGTGTGTTGTGTGTAATCTTCTCTC	300	4	0.62	0.67
OsNPR2/NH2	Os01g56200	(TC)9	5' UTR	F:5'CTTCGTCCTCGTCGTCTTCCTC R:5'GGGAAACGGATCTCGGAGAGAG	264	2	0.35	0.46
Cytochrome P450	Os03g40540	(GA)21	Intron	F:5'AGAGGCAGAGGAAGAAGGAGCTG Reverse 5'GCACTTTTTGGTGGACTGGAAGG	224	5	0.66	0.71
OsLOX1	Os03g49380	(GA)16	5'UTR	F:5'CGGCTTCGTCTCTCTCTCCATC R:5'CATCCTCGCAGATCACAAGTCAC	162	5	0.73	0.77
Pdk1	Os01g65230	(CT)14	5'UTR	F:5'CAAGGAAGACGACGAAGAGGAGG R:5'GTGGAGGAACCATCGGATTCG	207	3	0.16	0.18
OsAOC	Os03g32314	(AT)11	5'UTR	F:5'CAACCATTCCGTTCAGTGTGC R:5'ATTGCGGCATCTTTAGTTGGTC	223	10	0.85	0.87
OsbHLH65	Os04g41570	(GA)13	5'UTR	F:5'GCTGCACCGGCTAACTGTAGTG R:5'GACGAACACCATGCATACTCCA	173	7	0.79	0.82

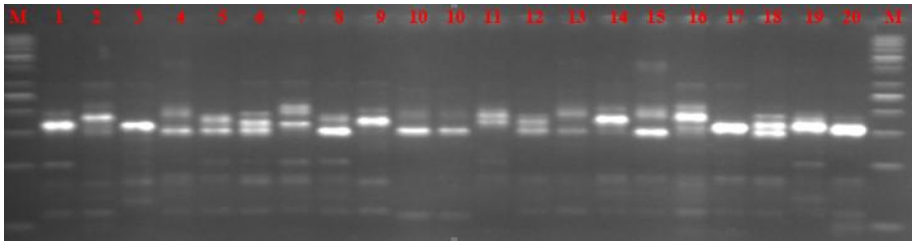

Fig 1: Banding pattern of lipoxygenase (LOC_Os03g49380) gene in the 18 two *O. sativa* cultivars and 18 *O. rufipogon* accessions (Lane M: 50bp ladder; 1-20: genotypes used in the study)

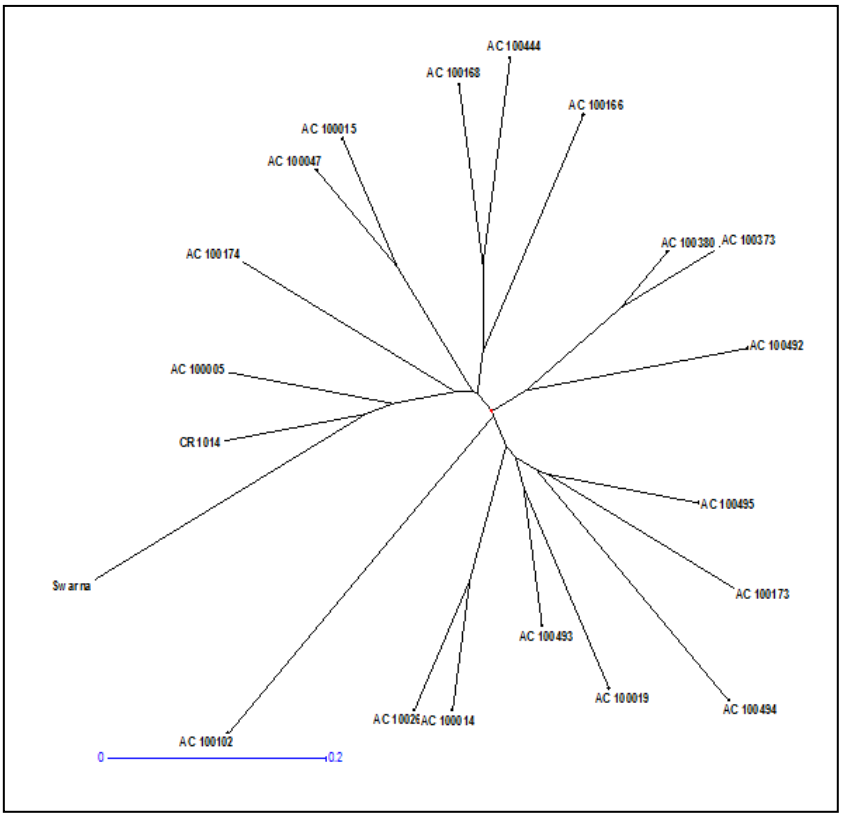

Fig 2: Dendrogram based on UPGMA clustering using 106 alleles of 21 cgSSR markers for 18 *Oryza rufipogon* accessions and two rice cultivars.

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