Publisher homepage: www.universepg.com, ISSN: 2663-6913 (Online) & 2663-6905 (Print)

https://doi.org/10.34104/ajpab.022.010021

American Journal of Pure and Applied Biosciences

Journal homepage: www.universepg.com/journal/ajpab

Genetic Analysis of Submergence Tolerance Rice Genotypes by Introgression of *Sub1* QTL to *Indica* HYV through Breeding Populations (F_2) with Marker Assay

Bishnu Pada Ray[1]*, Ujjal Kumar Nath[2], and Md. Abul Kalam Azad[3]

[1]Biotechnology division, Bangladesh Institute of Nuclear Agriculture (BINA) and GPB, BAU Mymensingh, Bangladesh; [2]Bangladesh Agricultural University, Mymensingh, Bangladesh; and [3]Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh.

*Correspondence: bpray2010@gmail.com (Bishnu Pada Ray, Senior Scientific Officer, Biotechnology division, BINA and GPB, BAU Mymensingh, Bangladesh).

ABSTRACT

More than 2.0 million-hectare area was affected by flash floods of various grades and reduced the average yield in Bangladesh by 5%. It involves introgression of *Sub1* QTL into the genetic background of HYV rice through marker-assisted breeding and to evaluate submergence tolerance of high yielding rice varieties. SSR profiling was performed to tag the submergence tolerant QTL by using *sub1* flanking markers and F_1 confirmation of Binadhan-7 x BRRI dhan52 (F_1) by using the primer RM1115. The gene diversity value was 0.7610 and the polymorphism information content (PIC) values were 0.7432 & allele frequencies (%) were 0.3328. Binadhan-7 x BRRI dhan52 (F_2) crosses were possessed the highest grain yield plant^{-1} (38.00 g) which was significantly higher than its both parents and also early maturing as 124 days from all crosses. The genetic similarity analysis using UPGMA (Unweighted Pair-Group Method using the Arithmetic Average) clustering system generated 5 major genetic clusters. Maximum intra-cluster degree of diversity was observed in cluster4 (79.93) and minimum in cluster3 (31.44). Highly significant and positive correlations were found among the grain yield (GY) and Total tillers/plant, effective tillers/plant, panicle length and filled grains/panicle. The first three principal components with Eigen values explained 73.7 % of the total variation among 16 rice genotypes for the 9 quantitative traits studied. However, it is hoped that promising *Sub1* cross combination Binadhan-7x BRRI dhan52 will be able to develop three to four weeks tolerance with high yielding submergence tolerant varieties to increase rice production in submerged prone areas of Bangladesh where single flash floods occur under different cropping patterns.

Keywords: DNA, Submergence, Marker, Yield, QTL, *Sub1,* Breeding populations, and PCR.

INTRODUCTION:

Rice (*Oryza sativa* L) has demonstrated the highest progresses in efficient genomics from the all crops in last recent decades. It has a small genome and diploid in nature comparison to any other cultivated cereals crop (Moin *et al.,* 2017). However rice genotypes parades light genetic variability due to repeated use of similar genotypes. Appreciative the genetic basis and the function of a particular gene can help for breeder to develop new and more productive with submergence tolerance genotypes. Extreme levels of flash flood were announced in the river basins of South and Southeast Asia. The estimated annual economic loss is more than US$ 600 million. Submergence especially

flash flood affected 15 million ha in South and South-East Asia region (Neeraja *et al.,* 2007).

Rice is only aquatic crops that are well developed aerenchyma tissues for that facilitate oxygen diffusion through continuous air spaces from shoot to root. However complete submergence due to frequent flash flood can adversely affected yield and other yield component. Rice is a main food in half of the world population and hence, is referred to as "Global Grain" (Prasad *et al.,* 2018; Kumar *et al.,* 2020) but its production chronologically decline because of submergence and other climate change. About more than 2.0 million ha land areas affected by flash flood and average yield about 5% loss in Bangladesh (Iftekharuddaula *et al.,* 2011; Ray *et al.,* 2018). Marker assisted selection (MAS) obviously proves the superiority by using MAB compared to conventional breeding because a small donor region only a few backcross population would be impossible using conventional methods (Ray *et al.,* 2014). For this reason we have to develop high yielding submergence tolerant genotypes and Marker assisted selection (MAS) can play a vital role to develop those genotypes. These researches will signifycantly assist to introgression of *Sub1* QTL into HYV rice cultivars through marker assisted breeding and evaluated high yield with highly tolerance to submergence. Submergence is the most challenging abiotic stress in the Rainfed Lowland Rice (RLR) ecosystem among the rice growing ecosystem. The *Sub1* QTL was about 70% on chromosome 9 of the phenotypic variation for survival under submergence has fine mapped on chromosome 9 and the cluster of genes underlying the QTL cloned (Karim *et al*., 2021; Xu *et al.,* 1996; 2000; 2006).

These QTL has introgressed into few HYV at the International Rice Research Institute (IRRI) (Iftekharuddaula *et al.,* 2011; Neeraja *et al.,* 2007; Septiningsih *et al.,* 2009; 2013; 2014) and Bangladesh Institute of Nuclear Agriculture (BINA). So far Bangladesh Rice Research Institute (BRRI) has been released two submergence tolerant varieties viz. BRRI dhan51 (Swarna-Sub1) and BRRI dhan52 (BR11-Sub1) and BINA has released another two submergence tolerant varieties viz. BINA dhan11 (Ciherang-Sub1) and BINA dhan12 (Samba Mahsuri-Sub1). These varieties can tolerate around two to three weeks of flash flood submergence. But Northern part of Bangladesh such as Rajshahi division and Rangpur division, duration of flash flood remain about 3 to 4 weeks. Now a days, already released varieties in Bangladesh cannot tolerate such type of stress level (3-4 weeks). Nonetheless, flash flood stress depends on duration submergence, turbidity, water temperature, pH, depth of water etc. With that case Mohanty *et al.* (2000) specified that submergence is polygenic trait and *Sub1* QTL does not alone completely represent this trait. Even with 3-4 weeks of submergence tolerance, current submerged varieties often damaged whenever environmental factors go beyond the tolerance of the current varieties except for the immersion period. That's why new submergence tolerant QTLs other than *Sub1* with the representation of agronomically suitable phenotypic variation need to be pyramided along with *Sub1* QTL. So far, very limited sources of submergence tolerance accessions were detected, and one of them coming from a moderately tolerant variety, IR72 (Septiningsih *et al.,* 2012). Many research studies have been recognized quantitative trait loci (QTLs) for submergence tolerance obtained from several populations (Nandi *et al.,* 1997; Siangliw *et al.,* 2003; Toojinda *et al.,* 2003; Xu and Mackill, 1996; Septiningsih *et al.,* 2012; Gonzaga *et al.,* 2016; Iftekharuddaula *et al.,* 2016). Among the discovered QTLs, the *Sub1* QTL from chromosome 9 derived from FR13A is known to be the most significant, contributing up to 2–3 weeks of submergence tolerance depending on the genetic background and the environmental conditions at the time of flooding. *Sub1* was previously fine mapped, the FR13Aderivedline was sequenced for the *Sub1* region, and a cluster of three ethylene response factor (ERF) genes, namely *Sub1a, Sub1b, and Sub1c*, were unveiled (Xu *et al.,* 2000; Xu *et al.,* 2006). *Sub1A* was found in a subset of *indica* and as accessions, while *Sub1b and Sub1c* were detected in all *indica* and *japonica* accessions screened for these genes (Xu *et al.,* 2006; Li *et al.,* 2010). Moreover, *Sub1A* was confirmed as the causal gene-providing submergence tolerance at the *Sub1* QTL (Septiningsih *et al.,* 2009; Singh *et al.,* 2010; Xu *et al.,* 2006). The activation of *Sub1A* under complete submergence stimulates the expression of the slend-errice-1 (SLR1) and SLR like-1 (SLRL1) genes through the suppression of ethylene consequently inhibiting gib-

berellic acid (GA)-mediated shoot elongation (Fukao and Bailey-Serres, 2008). Thereby starch and sugar of leaf consumption are intentionally slower compared to the intolerant lines lacking *Sub1*that quickly consume carbohydrate energy reserve for shoot elongation due to the activation of ethylene that signal GA accumulation. Hence, intolerant lines die after the water subsides because all the carbohydrate reserves were spent during submergence, while lines containing the tolerant *Sub1A* allele retain enough carbohydrate serves to recover after the water subsides (Fukao and Bailey-Serres, 2008) in **Fig. 1.**

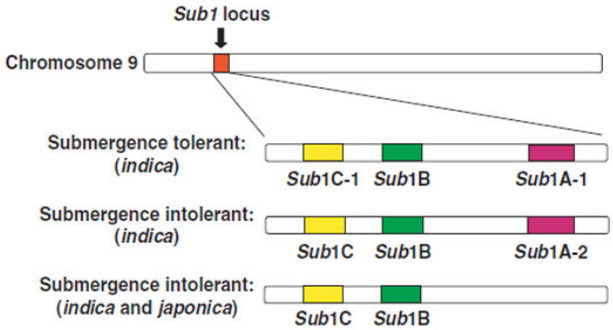

Fig. 1: *Sub1* locus in rice (*Oryza sativa L.*) (Devet al.2018, Fukao *et al.*, 2009).

MATERIALS AND METHODS:

Rice materials

This experiment consists of 16 genotypes among them two submergence tolerant genotypes as BRRI dhan52, FR13A that have *sub1*QTL and 3 susceptible HYV as Binadhan-7, Binadhan-17, BRRI dhan49 varieties and 4 swarna genotypes such as Guti swarna, Mamun swarna, Bilati swarna and Panpata swarna and 7 cross combinations as Binadhan-7 x BRRI dhan 52 (F_2), Bindhan-17 x BRRI dhan52 (F_2),Guti swarna x BRRI dhan52 (F_2), Panpata swarna x BRRI dhan52 (F2), Bilati swarnax BRRI dhan52 (F_2), Mamun swarna x BRRI dhan52 (F_2), BRRI dhan49 x FR13A(F_2). Data on dm= Days to maturity, ph= Plant height (cm), tt = Total tillers/plant, et= Effective tillers/plant, pl= Panicle length (cm), fg= Filled grains/plant, ug= Unfilled grains/plant, sw=Thousand seed weight (g), Yld= Yield/plant (g) were recorded.

Genotypic Analysis

The leaves segment (2-3 cm pieces) of every genotype for DNA extraction completed by using the Cetyl Trimethyl Ammonium Bromide (CTAB) mini-prep method (IRRI. 1997) at Biotechnology laboratory, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh Bangladesh. CTAB method described by Zheng *et al.* (1995) is modest and fast compared to the any other methods and no need liquid nitrogen (Ray *et al.,* 2016). DNA qualities of this method were enough for PCR**.** PCR were accomplished by using the procedure of Chen *et al.* (1997) and the PCR product were visualized on 1.5% agarose gels. After initial deformation for 2 min at 94°C, each cycle has a 30 second denaturation at 94° C temperature, 30 second annealing at 55° C, and final minute extension for 5 minutes at 72° C at the end of 34 cycles. The PCR product were mixed with bromophenol blue gel loading dye and were analyzed by electrophoresis on 6% polyacrylamide gel (PAGE) for the all SSR primers using mini vertical polyacrylamide gels for high throughout manual genotypes. The gels were stained in 0.5 mg/ml ethidium bromide and gel picture were taken using Molecular Imager gel documentation unit. SSR profiling were performed to tag the submergence tolerant QTL by using *sub1* flanking markers (**Table 7**)

Data analysis

SSR primers each allele size were measured according to the molecular weight by using Alpha-Ease 5.5 software. Allele size of the SSR markers were exam-ined using the program Power Marker version 3.25 (Liu and Muse, 2005). The statistic of the primers measured with the counting the numbers of alleles per locus, major allele frequency, gene diversity, polymorphism information content (PIC) values were analyzed by using this software. Genetic distant coefficient and a dendogram demonstrating the genetic relation between genotypes, based on the unweighted pair group method with arithmetic averages (UPGMA) were created by using same program and viewing in Tree View (MEGA software). The value of this data on dm= Days to maturity, ph= Plant height (cm), tt = Total tillers/plant, et= Effective tillers/ plant, pl= Panicle length (cm), fg= Filled grains/plant, ug= Unfilled grains/plant, sw= Thousand seed weight (g), Yld= Yield/plant (g) were recorded for each population of trial. Data analyses were done to calculate Analysis of variance (ANOVA), mean performance, correlation coefficients, Principal Components Analysis (PCA), Dendogram by using the software ige-

tintopc.com_Minitab_18.1 for yield and yields contributing characters of rice genotypes in Aman season, 2019. Data calculated on significance difference (P<0.001) that is *** = Significant at 0.01% level of probability, ** = Significant at 1% level of probability (P<0.01), * = Significant at 5% level of probability (P<0.05).

RESULTS:

The analysis of variance supported for nine characters existed in **Table 1** which showed that the genotypes differed significantly for that all same characters. Among the all parent genotypes the top mean value yield plant^{-1} (32.00gm) was produced by Panpata swarna and the bottom (21.00g) by Binadhan-17 and FR13A. On the other hand the group of Binadhan-7 x BRRI dhan52 (F2) crosses was higher yielding (38.00 g) than Guti swarna x BRRI dhan52 (F2) cross combination (22.00g). Binadhan-7 x BRRI dhan52 (F2) crosses were possessed the highest grain yieldplant^{-1}(38.00 g) which was significantly higher than its both parents (**Table 2**). Other high yielding cross combination of Bindhan-17 x BRRI dhan52 (F2) were 31.00 g and those were also significantly higher than their related parents. So, desired grain yeild plant^{-1}were found from those high yielding cross combination Binadhan-7 x BRRI dhan52 (F2). Binadhan-7 x BRRI dhan52 (F2) and Bindhan-17 x BRRI dhan52 (F2) cross combinations were early maturing also as 124 day and 125 day correspond (**Fig. 2**). It was an excellent observation that most of the high yielding combinations than their both parents. So, yield contributing gene(s) from that parent were transferred to the cross combinations successfully also. It was importantly noticed that the Binadhan-7 x BRRI dhan52 (F2) cross combination, highly significant heterosis over mid parent and better parent were also positively heterosis and significant in most case for the other yield contributing character or traits such as number of effective tillers plant^{-1}, filled grains panicle^{-1}. Among the all crosses, Binadhan-7 x BRRI dhan52 (F2) were excellent considering overall performance. Binadhan-7 x BRRI dhan52 (F2) expressed preferable performance in case of days to maturity and showed significant in some important yield contributing characters such as for crosses the highest effective tillers plant^{-1} (14.93) was observed in Binadhan-7 x BRRI dhan52 (F2) combination, where the value was in desired direction and more than its both parents. Effective tillers plant^{-1}, filled grains panicle^{-1}, thousand grain weight (g) also. Therefore, these cross combination may be recommended for further research with their control varieties to desirable lines with high yield capabilities, high sub-mergence tolerance with earliness.

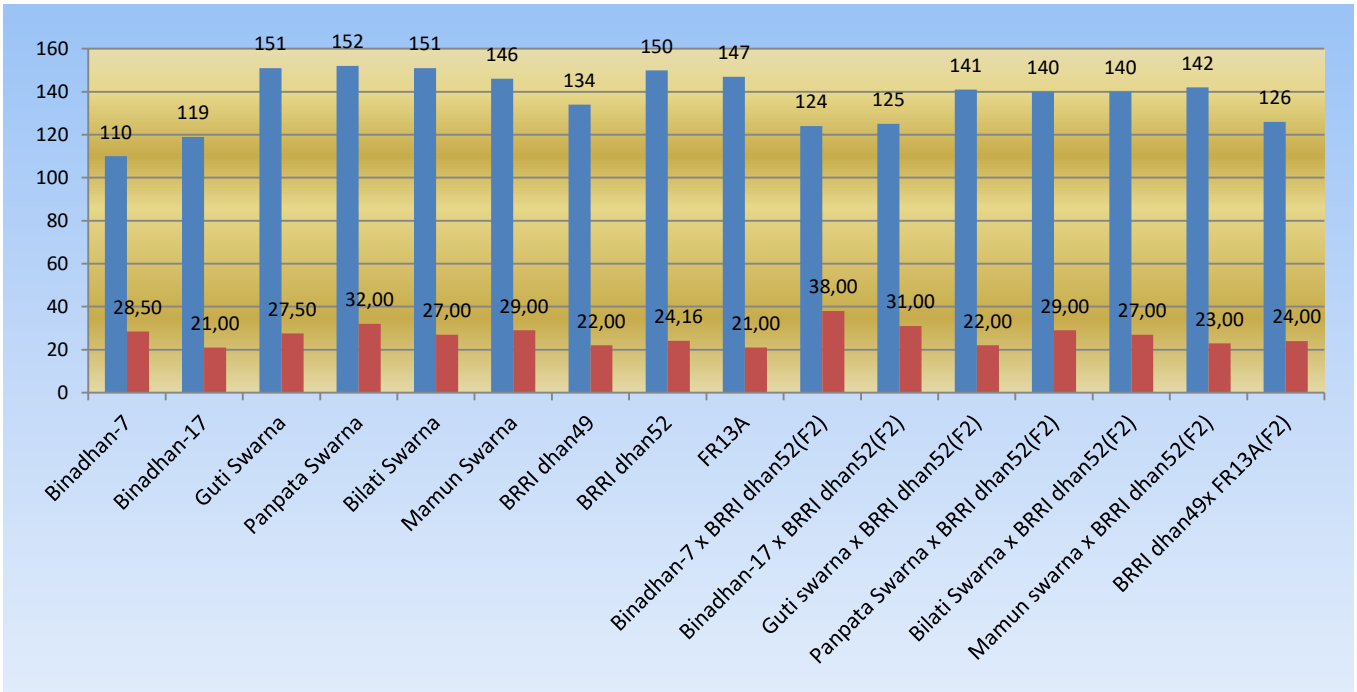

Fig. 2: Bar graphical presentation of yield with 16 rice genotypes for submergence tolerance genotypes.

Table 1: Analysis of variance (ANOVA) of parents and cross combinations (F2) for yield and yields contributing characters of rice genotypes in Aman season.

Source of variation	df	Mean sum of squares								
		dm	ph	tt	et	pl	fg	ug	sw	yld
Replication	2	15.06	5.64	7.96	15.015	3.26	16.00	9.09	15.30	16.09
Genotype	15	493.18***	212.23***	12.42***	9.129***	10.53**	1421.15*	798.37***	12.38***	65.33***
Error	30	0.06	1.10	0.62	0.028	0.607	0.00	3.810	0.011	0.0001

*** = Significant at 0.01% level of probability,

Here,

dm= Days to maturity, ph= Plant height (cm), tt = Total tillers/plant, et= Effective tillers/plant, pl= Panicle length (cm), fg= Filled grains/plant, ug= Unfilled grains/plant, sw=Thousand seed weight (g), Yld= Yield/plant (g).

Table 2: Mean performance of parents and cross combinations (F2) for grain yield plant^{-1}and yield contributing traits in Aman season.

Parents & crosses	Genotypes	gen	dm	ph	tt	et	pl	fg	ug	sw	yld
Parents	Binadhan-7	1	111 n	93.00 gh	14.66 bcd	14.00 b	24.33 cdef	120.00 m	19.66 f	23.00 c	28.50 e
	Binadhan-17	2	119 m	89.00 i	10.33 g	9.00 g	20.33 g	141.00 f	17.00 fg	21.49 e	21.00 k
	Guti swarna	3	151 b	112.93 b	17.40 a	15.00 a	28.40 a	130.00 j	41.33 bc	19.80 h	28.00 e
	Panpata swarna	4	152 a	99.00 de	14.66 bcd	13.00 c	25.66 bcde	140.00 g	31.66 e	20.00 h	32.00 b
	Bilati swarna	5	151 b	102.33 c	13.66 cdef	12.00 d	26.00 bcd	142.00 e	34.33 de	19.90 h	27.50 e
	Mamun swarna	6	146 e	101.20 cd	14.86 bc	12.06 d	24.36 cdef	160.00 b	30.46 e	20.90 f	29.00 d
	BRRI dhan49	7	134 i	102.00 cd	12.33 defg	11.00 e	24.00 def	120.00 l	22.33 f	17.15 i	22.00 j
	BRRI dhan52	8	150 c	111.86 b	13.33 cdef	12.00 d	26.53 abc	112.00 o	31.00 e	25.16 a	24.15 g
	FR13A	9	147d	104.33 c	12.00 efg	11.83 d	23.66 def	80.00 p	42.33 bc	21.00 f	21.00 k
Cross combinations	Binadhan-7 x BRRI dhan52 (F2)	10	124 l	96.86 ef	16.86 a	14.93 a	27.06 ab	132.00 i	12.86 g	24.00 b	38.00 a
	Bindhan-17 x BRRI dhan52 (F2)	11	125 k	97.66 ef	14.00 cde	13.00 c	23.00 f	170.00 a	47.13 b	23.09 c	31.00 c
	Guti swarna x BRRI dhan52 (F2)	12	141g	91.66 hi	11.33 fg	10.00 f	24.33 cdef	125.0 k	33.06de	23.30 c	22.00 j
	Panpata swarna x BRRI dhan52 (F2)	13	140 h	102.60 c	15.60 abc	13.66 b	25.66 bcde	135.00 h	43.73 bc	22.39 d	29.00 d
	Bilati swarnax BRRI dhan52 (F2)	14	140 h	95.73 fg	12.00 efg	11.00 e	25.93 bcde	148.00 d	38.26 cd	23.75 b	27.00 f
	Mamun swarna x BRRI dhan52 (F2)	15	142f	89.26 i	11.66 efg	10.00 f	24.50 cdef	155.00c	45.50 b	20.55 g	23.00 i
	BRRI dhan49x FR13A(F2)	16	126 j	118.20 a	15.33 abc	13.00 c	23.56 ef	112.00n	83.00 a	22.64 d	24.00 h

Dendrogram

UPGMA (Unweighted Pair Group Method of Arithmetic Mean)

A dendrogram constructed based on Nei's, (1983) genetic distance using UPGMA indicated segregation of 16 rice lines into two main groups. One of the main groups consists of two cluster, cluster4 and cluster5. Cluster4 contain one genotypes viz. FR13A and cluster5 contain BRRI dhan49 x FR13A (F2). Another main group consists of three clusters, cluster1, cluster 2 and cluster3. cluster3 consists of two sub clusters, sub cluster I consist of Mamun swarna, Bilati swarnax BRRI dhan52 (F2), Mamun swarna x BRRI dhan52 (F2) and sub cluster II consists of Bindhan-17 x BRRI dhan52 (F2) and Sub cluster IV consists of Cluster2 consists of two sub clusters, sub cluster III and sub

cluster IV. Sub cluster III consists of BRRI dhan49, BRRI dhan52, Guti swarna x BRRI dhan52 (F2), Sub cluster IV consists of Guti swarna, Panpata swarna, Bilati swarna, Panpata swarna x BRRI dhan52 (F2) (**Fig. 3**). Binadhan-7, Binadhan-17, Binadhan-7 x BRRI dhan52 (F2) genotypes are in the same cluster1. Based on above result, it may be concluded that, the maximum rice genotypes grouped in same cluster where the control genotype BRRI dhan52 is present and this may occur due to crosses and higher genetic distance (**Table 3**). According to the rang of diversity, five cluster were grouped for 16 genotypes. The distribution pattern revealed maximum number of genotypes (7 genotypes) in cluster 2 while cluster 4 and cluster 5 included minimum numbers of genotypes (1genotype). Maximum intra-cluster degree of diversity was observed in cluster4 (79.93) and minimum in cluster3 (31. 44). Maximum inter-cluster distance (74.56) was showed from cluster5 Minimum distance was found between the genotypes of the cluster3 (41.68) in **Table 4**.

Table 3: Number, percent and name of genotypes in different cluster (Final partition).

Cluster number	Number of genotypes	Percent (%)	Average Distance centroid	Maximum Distance From centroid	Name of genotypes
Cluster1	3	18.75	14.17	15.01	Binadhan-7, Binadhan-17, Binadhan-7 x BRRI dhan 52 (F2)
Cluster2	7	43.75	15.97	20.31	Guti swarna, Panpata swarna, Bilati swarna, BRRI dhan49,BRRI dhan52, Guti swarna x BRRI dhan52 (F2),Panpata swarna x BRRI dhan52 (F2)
Cluster3	4	25.00	13.87	19.59	Mamun swarna, Bindhan-17 x BRRI dhan52 (F2), Bilati swarnax BRRI dhan52 (F2), Mamun swarna x BRRI dhan52 (F2)
Cluster4	1	6.25	0.00	0.00	FR13A
Cluster5	1	6.25	0.00	0.00	BRRI dhan49 x FR13A (F2).

Table 4: Intra and inter-cluster average distance in 16 rice lines (Distances between cluster centroids).

	Cluster2	Cluster3	Cluster4	Cluster5
Cluster1	34.27	41.68	68.75	74.56
Cluster2		31.44	52.02	58.39
Cluster3			79.93	68.43
Cluster4				53.45

Note: Values in bold illustrate the intra-cluster distance and others show inter-cluster distance

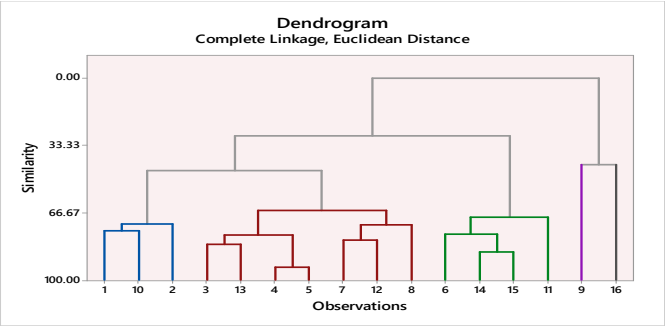

Fig. 3: UPGMA dendrogram showing the genetic relationship among rice genotypes in Aman season.

Here, 1=Binadhan-7, 2=Binadhan-17, 3=Guti swarna, 4=Panpata swarna, 5= Bilati swarna, 6=Mamun swarna, 7=BRRI dhan49, 8=BRRI dhan52, 9=FR13A, 10=Binadhan-7 x BRRI dhan 52 (F2), 11= Bindhan-17 x BRRI dhan52 (F2),12=Guti swarna x BRRI dhan52 (F2),13=Panpata swarna x BRRI dhan52 (F2), 14=Bilati swarnax BRRI dhan52 (F2), 15=Mamun swarna x BRRI dhan52 (F2), 16=BRRI dhan49 x FR13A(F2).

Correlations of Phenotypic Parameters

The correlation co-efficient between yield and yield contributing characters in rice are presented in **Table 5**. The correlations between the phenotypes of the parental lines and the crossing populations were also analyzed ($p < 0.05$ and 0.01). Highly significant and positive correlations were found among the grain yield (GY) and Total tillers/plant, effective tillers/plant, panicle length and filled grains/panicle. In addition, non-significantly but negative correlations were observed the grain width (GW) and days to maturity, plant height, unfilled grains/panicle. Grain yield was positively and non-significantly correlated with 1000 grains weight.

Table 5: Pearson correlation coefficients among different pairs of yield and yield contributing characters for different genotype of rice in Aman season.

	dm	ph	tt	et	pl	fg	ug	sw
ph	0.341*							
tt	0.041	0.504***						
et	-0.021	0.444**	0.878***					
pl	0.504***	0.369**	0.582***	0.598***				
fg	-0.047	-0.427**	0.049	-0.069	-0.036			
ug	0.146	0.555***	0.132	0.083	-0.044	-0.088		
sw	-0.240	-0.019	0.137	0.277	0.135	-0.006	0.060	
yld	-0.064	-0.002	0.706***	0.729***	0.501***	0.414**	-0.208	0.273

*** = Significant at 0.01% level of probability, ** = Significant at 1% level of probability, * = Significant at 5% level of probability.

Here, dm= Days to maturity, ph= Plant height (cm), tt = Total tillers/plant, et= Effective tillers/plant, pl= Panicle length (cm), fg= Filled grains/plant, ug= Unfilled grains/plant, sw=Thousand seed weight (g), Yld= Yield/plant (g).

Trait association

Principal component analysis

The PCA of nine yield and yield related traits and their other contrition characters with the total genetic divergence were presented in **Table 6**. According to the suggestion of Brejda *et al.* (2000), data were considered in each component with Eigen values more than 1, as it determines a minimum 10% of the variation. Superior Eigen values are considered as best attributes in principle components. A loading plot graphically showed the important PCs that explained major variability obtained by drawing a graph between Eigen values and principle component numbers (**Fig. 4**). The result of the study showed that three components indicated Eigen values of greater than one. The first three principal component with Eigen values explained 73.7 % of the total variation among 16 rice genotypes for the 9 quantitative traits studied. These first three principle components PC1, PC2 and PC3 explained 37.0 %, 22.3 % and 14.5 % of data variation, respectively (**Table 7**).The first principal component (PC1) accounted for more than 39.4% of total variance. The results showed that number of effective tillers/plant had the highest positive loadings (0.506), followed by number of total tillers/plant (0.504), panicle length (0.426), Grain yield per plant (0.409) plant height (0.310), Thousand seed weight (0.150), days to maturity (0.121) and number of unfilled grains/plant (0.088). Only one characters showed negative loading in the first principal component (PC1) that is number of filled grains/plant (-0.001) (**Fig. 4**). The second principal component (PC2) accounted for more than 22.3 % of total variance whereby plant height (0.514), number of unfilled grains/plant (0.433), days to maturity (0.360), panicle length 0.056)) exhibited positive loadings. On the contrary, yield/ plant (-0.415) total tillers/plant (-0.049), effective tillers/plant (-0.080), and. and thousand seed weight -0.201), number of filled grains/plant (-0.441), showed negative loading (**Table 6**).

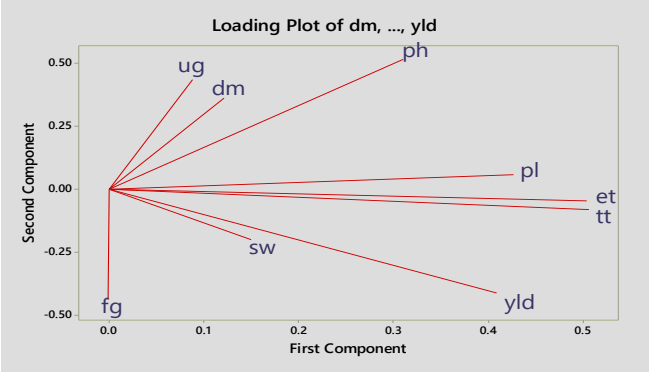

Fig. 4: Loading plot of principal component analysis of 16 rice genotypes for yield related traits.

The third principal component (PC3) accounted for more than 14.5 % of total variance and days to maturity 0.627) panicle length (0.354), number of filled grains/plant 0.285), yield/-plant (0.076) exhibited positive loading. Besides, these showed negative loading Plant height (-0.138). Unfilled grains per panicle (-0.308) thousand seed weight (-0.502) number of total tillers/plant (-0.063) and number of effective tillers/plant (-0.158).

Table 6: Principal components (PCs) for nine yield and yield related traits in 16 rice genotypes from principle component analysis with Eigen vectors (loadings) of the first three principal components.

Variable	PC1	PC2	PC3
Eigenvalue	3.32	2.00	1.30
Proportion (%)	37.0	22.3	14.5
Cumulative (%)	37.0	59.3	73.7
Days to maturity	0.121	0.360	0.627
Plant height (cm)	0.310	0.514	-0.138
Total tillers per plant	0.504	-0.049	-0.063
Effective tillers per plant	0.506	-0.080	-0.158
Panicle length (cm)	0.426	0.056	0.354
Filled grains per panicle	-0.001	-0.441	0.285
Unfilled grains per panicle	0.088	0.433	-0.308
Thousand seed weight (g)	0.150	-0.201	-0.502
Grain yield per plant (g)	0.409	-0.415	0.076

Marker analysis

Totally 56 simple sequence repeat (SSR) primer were hand-me-down for parental survey (IRGSP, 2005; Ma-Couch *et al.,* 2002; Temnkh *et al.,* 2001). All information of the SSR primers collected from the Gramene database (www.gramene.org). For the primer survey DNA band of the all genotypes as FR13A,BRRI dhan52,Guti swarana, Mamun swarna, Bilati swarna,Binadha-17, Binadhan-7 etc polymorphism were score according to their molecular weight on polyacrylamide gel electrophoresis. The band of the primer amplified at many level were measured as polymerphic marker but those primers which produced same level band that is called monomorphic marker. On the other hand, those primers which is not produced any types of band were considered as not amplified. Notably, water was used as negative control for every primer. This strategy is very helpful to identify fake bands produced from any type of pollution or unexpected nucleotides in primer or master mix.

For producing BC_1F_1 seeds F_1 seeds were disinfected by treating with 0.1% Bavistin 50SP solution for one hour. Confirmation of crosses was done by comparing F_1s with the parents as well as by marker assisted selection. At the same time backcrossing of F_1 plants with the recipient parent was done. Leaves collection of F_1 plants (1-14 plants) and their parents were completed for DNA extraction with marker assay. The F_1population confirmation of Binadhan-7 x BRRI dhan52 (F_1) were completed by using the primer RM 1115. Plants number 1, 4, 13, and 14 were not heterozygous band but plants number 2,3,5,6,7,8,9,10, 11,12 were heterozygous band that is F_1 confirmation were finished (**Fig. 5**). This confirmed F_1 goneto BC1F1 & F_2 generations. The amplicon size of all genotypes for RM1115 marker allele was measured and shown in **Table 7**. The number of alleles was 6 per locus. The gene diversity value was 0.7610 and the polymorphism information content (PIC) values were 0.7432 & allele frequencies (%) were0.3328.

Confirmation of F_1

Table 7: The sequence and position of submergence tolerant primer and number of alleles, allele size range, allele frequency (%) and Polymorphism Information Content (PIC) of 11 rice genotypes for SSR primer.

SN	SSR Marker	DNA Sequence	No. of base	Ann. Temp.	Expected product size (bp)	Position (bp)	Chromosome Number	Repeat Motif
1	RM1115	F-GCTGCAATTTATACCGGAGG R-AGCCACCACCATCTATCTGC	20 20	55	154	14,758,704-14,758,857	5	(AG)12

	Marker	Position	No. of allele	Allele Frequency (%)	Gen diversity	PIC	Chro. No.
1	RM1115	14,758,704-14,758,857	6	0.3328	0.7610	0.7432	5

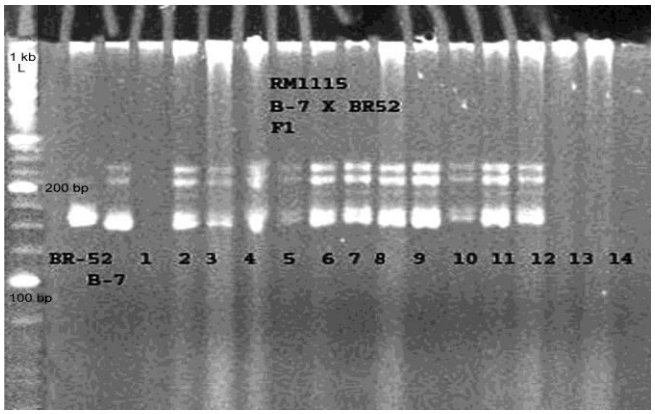

Fig. 5: DNA profile of RM1115 for F1 confirmation in the cross of Binadhan-7 x BRRI dhan52 (F1) in rice genotypes.

DISCUSSION:

Ahangar *et al.* (2008) found significant mean sum square for all the characters studied except 1000 grain weight. Average performance of the genotypes with two standard check varieties such as BRRI dhan51, BRRI dhan52 presented that lowest growth duration was obtained from BRRI dhan52 (148 days). Plant height from 94 to 120cm. The highest grain yield (4.85 t/ha) was obtained from BRRI dhan52 followed by BRRI dhan51 (4.6 t/ha). The survival percentage were more than 90% from all genotypes showed that there was not much stress of flash flood of this experiments (Ray *et al.,* 2013). Based on the above result, it may be concluded that, the highly tolerant and moderately tolerant germplasms grouped in different cluster due to higher genetic distance. The dendrogram also revealed that the genotypes that are derivative of genetically similar type clustered together. Tehrim *et al.* (2012) conducted a study with 35 rice cultivars and resolved a significant cluster analysis where most of the cultivars were close to each other showing a high level of genetic relatedness. Chakravarti *et al.* (2006) classified the rice genotypes into 11 distinct groups. Cluster analysis of the 193 accessions parental lines of rice showed 3 major groups and nine subgroups (Ren *et al.,* 2003). Lower levels of polymorphism in rice germplasms indicated that there is a basic similarity among the rice cultivars, which is to be expected due to their same ancestors and selection for similar characteristics. Vange *et al.* (2009), Yadav *et al.* (2010), Akinwale *et al.* (2011), Selvaraj *et al.* (2011) described also positive and significant correlation of yield with other yield contributing traits. Prasad *et al.* (2001) and Nandeswar *et al.* (2010) establish significant nut negative correlation of plant height with yield whereas Rangare *et al.* (2012) initiate non-significant and positive correlation of the traits with yield. But the findings of the experiments contradict with the results of Selvaraj *et al.* (2011) presented positive and significant correlation of yield with filled grain/panicle. Thousand grains weight (TGW) was negatively and significantly correlated with days to maturity and yield per plant. Similar result also obtained by Karad *et al.* (2008). These principal components have been seen contributing to 78.2632 percent per total variety (Kumar *et al.,* 2020). Similar results were reported earlier in rice by Gana *et al.* (2013) and Nachimuthu *et al.* (2014). Prasad *et al.* (2001) and Ali *et al.* (2010) were reported that the number of alleles per locus ranged from 2 to 8 with an average 3.8 using BRRI released varieties. The comparable studies is to three previous estimates of microsatellite analysis in rice viz., 0.26 to 0.65 with an average of 0.47 (Singh *et al.,* 2015), 0.28-0.50 with a mean of 0.45 (Umadevi *et al.* 2014) and 0.239 to 0.765 with an average of 0.508 (Hossain *et al.,* 2012).

CONCLUSION:

More than 2.0 million ha land area are affected by different types of flash flood and reduce average 5% reduces yield in Bangladesh. This is to introgression of *Sub1*QTL into the genetic HYV background rice by through marker assisted selection and to evaluate high yielding rice varieties with tolerant to submergence. SSR profiling were performed to tag the submergence tolerant QTL by using *sub1* flanking markers and F_1 confirmation of Binadhan-7 x BRRI dhan52 (F_1) by using the primer RM1115. Plants number 1, 4, 13, 14 were not heterozygous band but plants number 2,3,5,6,7,8,9,10,11,12 were heterozygous band that is F_1 confirmation were finished. Binadhan-7 x BRRI dhan52 (F2) crosses were possessed the highest grain yield$plant^{-1}$(38.00 g) which was significantly higher than its both parents and also early maturing as 124 day from all crosses. Therefore these cross combination may be suggested for further research with check varieties to select desirable genotypes or segregates with higher yield potentiality, earliness and other important characters.

ACKNOWLEDGEMENT:

I want to give very special thanks are due to my colleagues in the workplace who were there to help me in every moment of this huge work. Chemicals and instruments are supported by BINA and GPB, BAU.

CONFLICTS OF INTEREST:

The authors declare that there is no conflict of interest

REFERENCES:

1) Ali MS, Hoque ME, Rabbi SMHA *et al.,* (2010). Genetic diversity of BRRI released rice varieties using microsatellite Markers. In: Poster presentation abstract. 3rd *Inter. Rice Cong. Hanoi, Vietnam*. pp. 46.
2) Ahangar, L., Ranjbar, G.A. and Nouroozi, M. (2008). Estimation of combining ability for yield and yield components in rice (*Oryza sativa* L.) cultivars using diallel cross. *Pakistan J. Biol. Sci,* **11**(9), 1278-1281. http://dx.doi.org/10.3923/pjbs.2008.1278.1281
3) Akinwale, M. G., Gregorio, G., Nwilene, F., *et al.,* (2011). Heritability and correlation coefficient analysis for yield and its components in rice (*Oryza sativa* L.). *Afr. J. Plant Sci.,* **5**(3), 207-212.
4) Brejda, j.j., Karlen, D.L., Smith, J.L. and Allan, D.L. (2000). Identification of regional soil quality factors and indicators II. Northern Mississippi Loess Hills and Palouse Prairie. *Soil Science Society of American Journal*, **64**(6), 2125-2135. https://doi.org/10.2136/sssaj2000.6462125x
5) Chakrabarthi, B. K., R. Naravaneni, (2006). SSR Marker Based DNA Fingerprinting and Diversity Study in Rice (*Oryza sativa* L). *African J. Biotech*, **5**(9), 684-88.
6) Chen X, Temnykh S, Xu Y, Cho YG, McCouch S.R., (1997). Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.), *Theor. Appl. Genet.,* **95**, 553–567. https://doi.org/10.1007/s001220050596
7) Fukao T, Bailey-Serres J., (2008). "Submergence tolerance conferred by *Sub1A* is mediated by *SLR1* and *SLRL1* restriction of gibberellin responses in rice", *Proceedings of the National Academy of Sciences of the United States of America,* vol.**105**, pp.16814–16819.
8) Gana AS, Shaba SZ, Tsado EK., (2013). Principal component analysis of morphological traits in thirty-nine accessions of rice (*Oryza sativa L.*) grown in a rainfed lowland ecology of Nigeria. *J. of Plant Breeding and Crop Science,* **5**,120-126. https://doi.org/10.5897/JPBCS2013.0381
9) Gonzaga ZJ, Carandang J, Sanchez DL., *et al.,* (2016) Mapping additional QTLs from FR13A to increase submergence tolerance beyond SUB1. *Euphytica,* **209**, 627–636.
10) Hossain, M.M., Islam, M.M., Hossain, H., *et al.,* (2012). Genetic diversity analysis of aromatic landraces of rice (*Oryza sativa L.*) by microsatellite markers. *Genes, Genomes and Genomics,* **6**, 42-47. https://doi.org/10.5455/faa.298103
11) Iftekharuddaula, K.M., Newaz, M.A., Salam, M.A., *et al.,* (2011). Rapid and high-precision marker assisted backcrossing to intro gress the SUB1QTLintoBR11, the rain fellow and rice me gavariety of Bangladesh. *Euphytica,* **178**, 83–97.
12) Iftekharuddaula, K.M., Ahmed, H.U., Ghosal, S., Amin, A., E.M., (2016). Development of early maturing submergence-tolerant rice varieties for Bangladesh. *Field Crops Res.,* **190**, 44-53. http://dx.doi.org/10.1016/j.fcr.2015.12.001
13) IRGSP. (2005). The map-based sequence of the rice genome. *Nature,* **436**, 793–800.
14) Karim MR, Islam M, Bhuiyan MSH, and Mian JA. (2021). Potassium fractions and rice yield in different agro-ecological zones of Bangladesh. *Am. J. Pure Appl. Sci*., **3**(2), 35-41. https://doi.org/10.34104/ajpab.021.035041
15) Kumar S. S., Suneetha Y., Sandeep R.D., and Srinivas T.(2020). Principal component analysis for yield and quality traits of coloured rice (*Oryza sativa L.*). *The Pharma Innovation J.* **9**(7), 456-462. https://doi.org/10.22271/tpi.2020.v9.i7g.4972
16) Li Z-X, Septiningsih EM, Quiloy-Mercado SM, McNally KL., (2010) Identification of Sub1A alleles from wildrice Oryza rufipogon Griff. *Genet Resour Crop Evol,* **58**, 1237–1242.
17) Liu, K, S V Muse. (2005). Power Marker: Integrated analysis environment for genetic marker data. *Bioinformatics*, **21**, 2128–2129. http://dx.doi.org/10.1093/bioinformatics/bti282

18) McCouch, S. R., Teytelman, L., Xu, Y. B., *et al.,* (2002). Development and mapping of 2240 new SSR markers for rice (*Oryza sativa L.*). *DNA Res,* **9**(6), 199–207. https://doi.org/10.1093/dnares/9.6.257
19) Mohanty, H. K., Mallik, S. and Grover, A., (2000). Prospects of improving flooding tolerance in lowland rice varieties by conventional breeding and genetic engineering. *Curr. Sci*., **78**, 132–137.
20) Moin M., Bakshi A., Saha A., Datta M., Kirti P.B., (2017). Gain-of-function mutagenesis approaches in rice for functional genomics and improvement of crop productivity. *Briefings in Functional Genomics.* https://doi.org/10.1093/bfgp/elw041
21) Nandeshwar BC, Pal S, Senapati BK, De DK., (2010). Genetic variability and character association among biometrical traits in F2 generation of some Rice crosses. *Electron. J. Plant Breed.* **1**(4), 758-763.
22) Nandi S, Subudhi PK, Senadhira D, Manigbas NL, *et al.,* (1997). "Mapping QTLs for submergence tolerance in rice by AFLP analysis and selective genotyping" *Molecular and General Genetics,* **255**, pp.1–8. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8499/
23) Neeraja, C. N., Rodriguez, R. M., Pamplona, A., (2007). A marker-assisted backcross approach for developing submergence-tolerant rice cultivars. *Theor Appl Genet*., **115** (6), 767-776. http://dx.doi.org/10.1007/s00122-007-0607-0
24) Nachimuthu VV, Robin S, Sudhakar D, *et al.,* (2014). Evaluation of rice genetic diversity and variability in a population panel by principal component analysis. *Indian Journal of Science and Technology*, **7**(10), 1555-1562.
25) Prasad RKK, Suneetha Y, Srinivas T. (2018). Genetic diversity studies in rice (*Oryza sativa L.*). *Electronic Journal of Plant Breeding*, **9**(4), 1335-1341. https://www.ejplantbreeding.org/index.php/EJPB/article/view/2937
26) Prasad, P.V.V., Craufurd, P.Q., Kakani, V.G., *et al.,* (2001). Influence of high temperature during pre- and post-anthesis stages of floral development on fruit-set and pollen germination in peanut. *Aust. J. Plant Physiol,* **28**, 233–240.P
27) Rangare NR., Krupakar A., Ravichandra K., *et al.,* (2012). Estimation of characters association and direct and indirect effects of yield contributing traits on grain yield in exotic and Indian rice (*Oryza sativa L.*) germplasm. *International Journal of Agricultural Science*, **2**(1), 54-61. http://www.inacj.com/attachments/sect...
28) Ray B.P., (2018). Genetic analysis and development of submergence tolerance rice (*Oryza sativa l.*) lines through MAS. *Int J Complement Alt Med (USA),* **11**(4), 244–249. https://doi.org/10.15406/ijcam.2018.11.00406
29) Ray, B.P., Rahman, K.N.M., Hossain M.E., Sarker, P.C., (2016). DNA extraction protocol without liquid nitrogen of cold tolerant rice. *World J.Biol.Medi.Science,* **3**(3), 59-67.
30) Ray, B.P., Iffterharuddaula, K.M., Ghosal, S., (2014). Marker Assisted Backcross for the development of Submergence Tolerant Rice (*Oryza sativa L*). *J. Biol. Chem. Research,* **31**(1), 01-05. https://doi.org/10.1007/s00122-007-0607-0
31) Ray, B.P., Sarker, S.k., Sarker, M., (2013). Genotype Selection and Selection Criteria of Submergence Tolerant Rice (*Oryza sativa L.*). *J. Biol. Chem. Research*, **30**(2), 409-420.
32) Ren, F., Br. Lu, S. Li, J. Huang, and Y. Zhu. (2003). A comparative study of genetic relationships among the aa-genome *Oryza* species using RAPD and SSR markers. *Theor. Appl. Genet.,* **108**(1), 113-20. https://doi.org/10.1007/s00122-003-1414-x
33) Selvaraj, C. I., Nagarajan, P., Thiyagarajan, K. and Bharathi, M. (2010). Predicting the relationship between molecular marker heterozygosity and hybrid performance using RAPD markers in rice (*Oryza sativa* L.). *Afr. J. Biotech.,* **9**(45), 7641-7653. https://doi.org/10.5897/AJB10.1128
34) Septiningsih, E.M., Pamplona, A.M., Sanchez, D.L., (2009). Development of submergence tolerant rice cultivars: the Sub1 locus and beyond. *Ann. Bot.,* **103**,151–160. https://doi.org/10.1093/aob/mcn206
35) Septiningsih, E.M., Collard, B.C.Y., Heuer, S., *et al.,* (2013). Applying genomics tools for breeding submergence tolerance in rice. In: Var-

shney, R.K., Tuberosa, R. (Eds.), Translational Genomics for Crop Breeding: Improvement for Abiotic Stress, Quality and Yield Improvement, **2**, *Wiley-Blackwell,* USA, pp. 9–30.

36) Septiningsih, E. M., Hidayatun, N., Sanchez, D. L., (2015). Accelerating the development of new submergence tolerant rice varieties: the case of Ciherang-Sub1 and PSB Rc18-Sub1. *Euphytica*, **202**(2), 259-268. https://doi.org/10.1007/s10681-014-1287-x
37) Singh, A., Saini, R., Singh, J., Arya, M., Ram, M. and Singh, P.K. (2015). Genetic diversity studies in rice (*Oryza Sativa L.*) using micro-satellite markers. *International J. of Agriculture, Environment & Biotechnology,* **8**: 143-152. https://doi.org/110.5958/2230-732X.2015.00019
38) Singh N, Dang TTM, Vergara GV, Pandey DM, (2010). Molecular marker survey and expression analysis of the rice submergence tolerance gene *SUBA, Theor. & Appl. Gen.,* **121**, pp. 1441-1453.
39) Siangliw, M., Toojinda, T., Tragoonrung, S. *et al.,* (2003). Thai jasmine rice carrying QTLch9 (*SubQTL*) is submergence tolerant. *Ann. Bot*., **91**, 255–261. https://doi.org/10.1093/aob/mcf123
40) Temnykh, S., DeClerck, G., Lukashova, A., *et al.,* (2001). Computational and experimental analysis of microsatellites in rice (*Oryza sativa L.*) frequency, length variation, transposon associations, and genetic marker potential. *Genome Res.,* **11**, 1441–1452.
41) Tehrim, S, Z H Pervaiz, M Y Mirza, *et al.,* (2012). Assessment of phenotypic variability in rice (*Oryza sativa L.*) cultivars using multivariate analysis. *Pak. J. Bot,* **44**, 999 - 1006. https://www.cabdirect.org/cabdirect/abstract/20123220260
42) Toojinda T, Siangliw M, Tragoonrung S and Vanavichit A., (2003). "Molecular genetics of submergence tolerance in rice: QTL analysis of key traits", *Annals of Botany,* **91**, pp.243–253
43) Umadevi, M., Veerabadhiran, P. and Manomani, S. (2014). Assessment of genetic diversity of rice (*Oryza sativa*) cultivars using simple sequence repeat (SSR) markers. *African Journal of Biotechnology,* **13**, 3547-3552. https://doi.org/10.5897/AJB12.1406
44) Xu, K., & Mackill, D.J., (1996). A major locus for submergence tolerance mapped on rice chromosome 9. *Mol. Breed,* **2**,219–224.
45) Xu, K., Xia, X., Fukao, T., Canlas, P., Rodriguez, R. M., Heuer, S., (2006). Sub1A is an ethylene response factor-like gene that confers submergence tolerancetorice. *Nature,* **442**, 705. https://doi.org/708. 10.1038/nature04920
46) Xu, K., Xu, X., Ronald, P.C., Mackill, D.J., (2000). A high-resolution linkage map in the vicinity of the rice submergence tolerance locus Sub1. *Mol. Gen. Genet.,* **263**(4), 681–689. https://doi.org/10.1007/s004380051217
47) Yadav, S. K., Suresh, B. G., Pandey, P. and Kumar, B. (2010). Assessment of genetic variability, correlation and path association in rice (*Oryza sativa L.*). *J. boi-sci.,* **18**, 1-8. https://doi.org/10.3329/jbs.v18i0.8767
48) Zheng K, Huang N, Bennett J, Khush GS. (1995). PCR-based marker-assisted selection in rice breeding, IRRI Discussion Paper Series No. 12, *International Rice Research Institute*, Los Banos, Phillipines.

Citation: Ray BP, Nath UK, and Azad MAK. (2022). Genetic analysis of submergence tolerance rice genotypes by introgression of *Sub1* QTL to *Indica* HYV through breeding populations (F2) with marker assay. *Am. J. Pure Appl. Sci.*, **4**(1), 10-21. https://doi.org/10.34104/ajpab.022.010021