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Determination of Adaptive Mechanisms for Flash Flooding Tolerance in Nepalese Cultivated Rice Genepool based on Morpho-physiological and Molecular Analysis

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Abstract: Flooding is notorious to rice (*Oryza sativa* L.) cultivation at any stages threatening the life of >100 millions people in Asia annually. Compare to other cereals rice has unusual capacity to tolerate both short and long term flooding attributed to coordinated efforts of several morphological, anatomical, biochemical and action of adaptive genes. In this study three hundred and thirteen Nepalese rice landrace collected from diverse geographic regions were completely submerged for 14 days. Status of *Sub1A* gene was monitored in tolerant rice accessions identified here. Diverse rice accession possessed differential elongation growth response and their survival ability ranged from 0-100%. Some of the rice accessions performed better than the tolerant check; FR13A. Monitoring of *Sub1A* gene presence revealed that some of the tolerant rice accessions are lacking the *Sub1A* gene and *SublA-1* allele. Four accessions; Bhaatsaar, Kariyaparewa pakha, Sauthari and Karangi possessed the better survival response than the FR13A and remaining accessions had poorer response. Among tolerant accessions; Kariyaparewa pakha and Sauthari were lacking the *Sub1A-1* allele though followed the quiescence growth response. Interestingly, eight rice accessions with *Sub1A-1* allele were found to be intolerant to 14 days of complete submergence. Unlike FR13A all the tolerant and intolerant rice accessions displayed the bleaching of chlorophyll pigment resulting the lower SPAD reading. The detailed morpho-physiological and molecular analysis unveiled that both tolerant and intolerant rice accessions harbored *Sub1A* gene and adapt the both quiescence and escape strategies in response to flash flooding. Beside *Sub1A* gene, there might be participation of other stress responsive factors that probably functions in close coordination with *Sub1* haplotype. In conclusion, *Sub1A-1* alone is not a major contributing factor to confer submergence tolerance in diverse rice accessions, thus haplotype based profiling followed by cloning and sequencing are suggested.

Key words: Elongation, hypoxia, quiescence growth, *O. sativa*, *Sub1A*, submergence

INTRODUCTION

World rice (*Oryza sativa* L.) production is limited largely by several forms of climate change directed environmental stresses, of which flooding induced submergence is a major constraint worldwide (Xu *et al*., 2006). Annually about 16% of the world rice areas are unfavorably submerged and affecting the livelihood of more than 100 millions people (Hossain and Abedin, 2004). Rice is unusually adapted to semiaquatic environments because of its well-developed aerenchyma tissues, that facilitate oxygen diffusion through continuous air spaces from shoot to root and avoid O_2 deficiency in roots. However, complete submergence due to frequent flooding can adversely affect the plant growth and yield (Fukao *et al*., 2006; Xu *et al*., 2006). In some of the rainfed paddy areas, the levels of flood water rise progressively during the rice growing season and can reach up to 7 meters whereas in others, flash flooding can fully submerge the plants for a few days to weeks (Voesenek and Bailey-Serres, 2009). Submergence especially limits the oxygen diffusion rate by 10^{-4} fold slower than in air resulting anaerobic metabolism and energy crisis (Bailey-Serres and Voesenek, 2008; Licausi and Perata, 2009). As a result, most of the flood-prone areas are planted with five times less yielding landraces that display a remarkable ability to adapt either in deepwater or in flash flooding conditions (Voesenek and Bailey-Serres, 2009). These traditional rice varieties are tolerant to flooding stress due to various adaptive mechanisms to survive periods of hypoxia or anoxia (Mackill *et al*., 1996; Hattori *et al*., 2009). An adaptation to submergence involves alterations in molecular, biochemical, physiological, genetical and

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anatomical/ morphological attributes in rice plants. These include energy generation through fermentative metabolism, aerenchyma development in parenchymal tissues that improves access to O_2, activation of ethylene promoted gibberellin (GA) mediated internode elongation in deepwater rice to shootup the foliage above the water surface for gas exchange and restricting growth and conserving precious energy until floodwater recedes in lowland rice (Bailey-Serres and Voesenek, 2008; Hattori *et al*., 2009). These abilities have enabled the rice crop to cultivate worldwide ranging from rain-fed and irrigated lowlands to deepwater (Khush, 1997).

Recent discovery indicated that both quiescence and escape adaptive mechanisms displayed by different rice accessions are under genetic control (Xu *et al*., 2006; Hattori *et al*., 2009; Lee *et al*., 2009) and explicated in terms of the major *Sub1* QTLs and qTIL12 (*SK1* and *SK2*), respectively (Xu *et al*., 2006; Hattori *et al*., 2009). These QTLs, were identified in two different ecotypes of rice cultivated in different water regime, encode different subgroup of ERFs (Ethylene Responsive Factors) genes whose expression is activated by plant hormone ethylene. Analysis of near-isogenic and transgenic lines confirmed that *Sub1A-1* restricts the escape strategy whereas *SNORKELs* trigger antithetically through increase GA production and responsiveness and enable the rice plant to survive in deepwater conditions. Submergence induced ethylene activates *Sub1A-1*, limiting ethylene production by feedback mechanism and promoting GA repressors SLR1 and SLRL1 (Fukao and Bailey-Serres, 2008; Nagai *et al*., 2010). Recent findings based on comparative microarray and metabolite studies also confirmed that *Sub1A-1* regulates numerous transcription factors associated with stress tolerance responses, supporting the past speculation based on physiological study (Jung *et al*., 2010). Likewise mechanisms of seed germination and underwater seedling growth in the flooded paddy has also recently revealed in which tolerance is governed by *CIPK15* gene encoding Calcineurin B-like-interacting protein kinases (Lee *et al*., 2009). All three adaptive genes express independently of each other and are considered crucial to cope the different types of flooding stress in the rice field. Despite these knowledges on adaptive mechanisms at the gene and protein level, our understanding of the diversity in mechanisms by which different rice accessions survive under varying nature of complete submergence is still to be discovered. Moreover, the potentiality of local Nepalese rice accessions has not been assessed yet against the flash flooding. Therefore, this study was undertaken to dissect the morpho-physiological and molecular mechanism using diverse germplasm representing all Nepalese rice ecosystem. In this research, we tried to elucidate the underlying diverse mechanisms that facilitate the rapid recovery of rice seedlings after flash flooding.

MATERIALS AND METHODS

Seeds of 313 rice accessions, collected from different parts of the country was reterived from National Genetic Resource Center, Nepal Agricultural Research Council (NARC), comprising landrace from Terai, Hills and Mountains were subjected to submergence experiment. For the screening of submergence tolerant plants, seeds of each rice accession were surface sterilized and soaked over night in Petri dishes containing sterile water wetted filter paper. Petridishes containing seed were left for 2-3 days in dark with a temperature of 28±2°C for germination. Approximately 120 pre-germinated caryopses were sown in two rows in 5 kg plastic tray ($0.38\times0.27\times0.07$ m^3) supplied with puddled soil without fertilizers. Each tray consisted of six accessions and performed the experiment in CRD (Completely Randomized Design) with three replications (Fig. 1a). Seedlings were allowed to grow for 14 days at 28±2°C with a 12 h photoperiod. Seedlings when reached the age of 12 days they were thinned to 50 seedlings/row. Fourteen days old seedlings were completely submerged in a 3,300 l water tank ($2.44\times1.22\times1.12$ m^3) for another 14 days (Fig. 1b). Throughout the experiment the water depth was maintained at 1.05 m. The survival ability was scored after 7 days of desubmergence and survival percent over universal check (FR13A) was calculated as: Survival % over check = (% survival of accessions/% survival of check)×100.

Fourty-four accessions were selected based on their survival percentage (>90 %) and quiscence growth during preliminary glasshouse screening. To determine the molecular mechanism underlying the tolerance in those selected accessions, presence of *Sub1A* gene was monitored using six sets of gene specific primers as described previously (Xu *et al*., 2006). To verify the results of preliminary screening and *Sub1A* monitoring, the accessions with and without *Sub1A* but having more than 90% survival ability were further subjected to air versus submergence experiment following CRD with three replications, 15 seedlings/replication in 2 kg plastic pots including three rice varieties; FR13A, Goda Heenati and Kurkaruppan as tolerant and Nipponbarre (*japonica*) as intolerant control. Due to the insufficient amount of seeds, 16 rice accessions were excluded from this experiment. Thus, this confirmatory (pot) experiment only consisted of 28 rice accessions. Height of the seedling

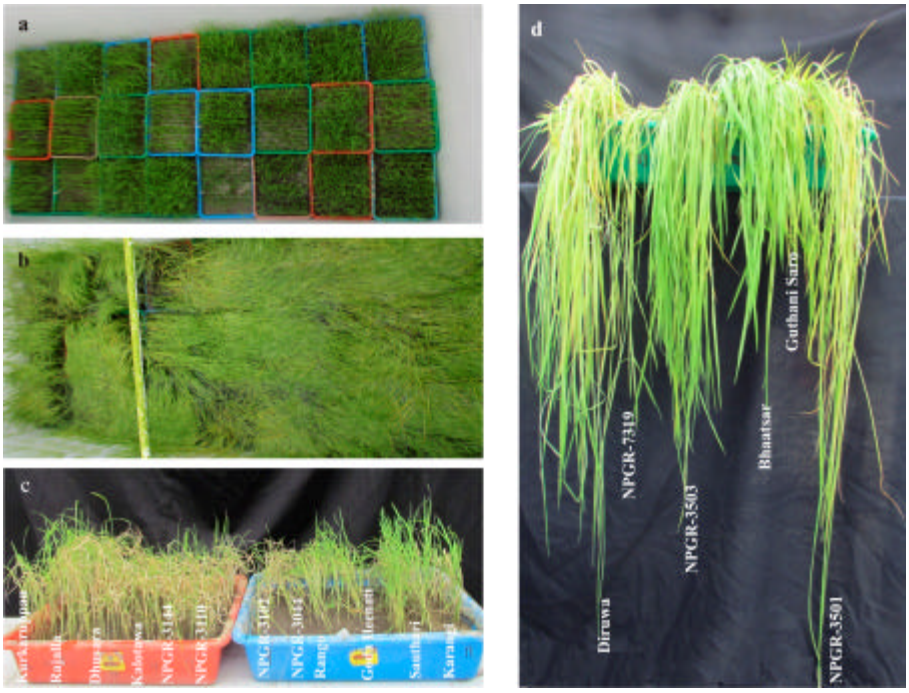

Fig. 1(a-d): Initial screening of different rice accessions against flash flooding. (a) 14 d old rice seedlings from different accession prior to submergence are laid out in CRD experiment inside the water tank, (b) morphological response of rice seedlings after submergence, (c) differential survival response of different rice accessions after desubmergence followed by 7 d recovery and (d) differential elongation response showing both quiescence and escape strategy adapted by different rice genotypes

and SPAD reading (SPAD 502, Minolta, Japan) were recorded from five randomly choosen seedlings/replication before and just after desubmergence to determine the elongation ability and total chlorophyll content. SPAD reading was recorded from three positions of a leaf. Survival ability of individual accession was scored after 14 days of submergence followed by 7 days of recovery.

Genomic DNA of rice accessions was prepared using modified CTAB method as described by Sul and Korban (1996). PCR reaction was conducted in the 15 μL volume containing 2 μL (100 ng) of genomic DNA, 1.5 μL (1 μM) of each primer, 7.5 μL of 2x GoTaq Green PCR Master Mix (Promega Corporation, Madison, WI, USA) and 2.5 μL PCR H_2O. The reaction mixture without template DNA (12.5 μL) was dispensed in each PCR tube. Finally, the DNA template from respective rice accessions was added in PCR reaction. PCR mixture was amplified in a MJ Research PTC-100 ™ Programmable Thermal Controller (MJ Research, Inc, Watertown, MA, USA.) with the following temperature regimes: initial denaturation for 2 min at 95°C followed by 32 cycles of 95°C for 30 sec, annealing at 56-60°C depending on the primers TM for 1 min, extension at 72°C for 2 min and final extension at 72°C for 7 min followed by holding at 4°C as described by Xu *et al*. (2006). Amplified PCR products were separated in 2% analytical grade agarose gel (Promega Corporation, Madison, WI, USA) using horizontal gel electrophoresis unit in 1×TAE (0.11% Glacial Acetic acid, 0.5 M EDTA and 0.04M Tris base) buffer and run at 100v for 1 h. Gels were stained with 0.1 μg mL^{-1} ethidium bromide (Promega Corporation, Madison, WI, USA) and then visualized under UV trans illuminator gel documentation system (Wilber Lourmat, Marne-La-Valleen, France.) using 1 μg guide size DNA ladder (Genetix, Biotech Asia Pvt. Ltd). Mean and standard deviation for height and chlorophyll contents and percentage of survival over check were computed using MS EXCEL Software (2007). Monitoring of *Sub1A* was done based on the presence/absence of the band in the respective lane of the accessions.

RESULTS

Three hundred and thirteen Nepalese rice landrace collected from diverse geographic regions were

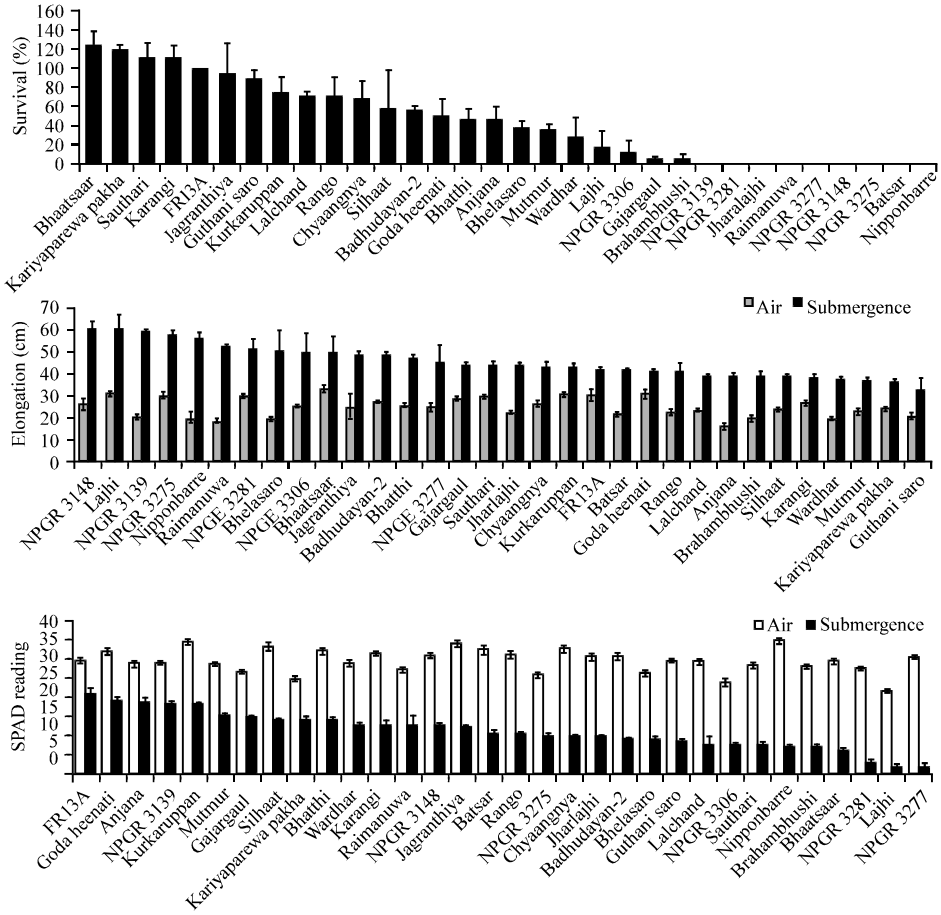

Fig. 2(a-c): Morphological and physiological response of different rice accessions after 14 days of complete submergence. (a) Survival response of selected rice accessions (with and without *Sub1A* gene) after 7 d of recovery. Mean and standard deviation were calculated from three replications with 15 seedlings/replication and is presented in the graph, (b) The elongation growth response of rice seedlings under 14 d of complete submergence. Both quiescence and escape adaptive mechanisms are shown in the graph. Data were collected as described earlier, (c) Graph showing SPAD reading to determine the chlorophyll content before and after submergence

completely submerged for 14 days. Depending upon the genetic makeup, these accessions possessed differential growth response and survival ability (Fig. 1c-d). In the initial screening, the submergence tolerance response was varied from 0-100% (Table 1) and some of the accessions were better than tolerance check; FR13A (Table 1, Fig. 2a, 3). Based on the result of large scale screening, 44 tolerant rice accessions were selected for further experiment without knowing the molecular mechanisms behind the tolerance. To know the molecular regulation of the tolerance in those selected rice accessions, the presence of major genetic regulator; *Sub1A* gene was monitored using *Sub1A* gene and *Sub1A-1* allele specific primer and found that some of the tolerance rice accessions lacking both *Sub1A* gene or *Sub1A-1* allele (Table 2, Fig. 2a). Though 44 rice accessions were selected for *Sub1A* presence monitoring, unfortunately high stringent submergence experiment using all accessions could not be performed due to lack of adequate number of rice seeds. Therefore, the comparative high throughput screening was restricted to only 28 accessions. In the repeated experiment some of the rice accessions found to be tolerant in earlier experiment showed the intolerancy (Fig. 2a). Four accessions; Bhaatsaar, Kariyaparewa pakha, Sauthari and Karangi possessed the better survival response than the FR13A and rest of the accessions had poorer response. Among tolerant accession; Kariyaparewa pakha and Sauthari were lacking the *Sub1A-1* allele and also followed the quiescence growth response (Fig. 3a, b, 2b).

Table 1: List of rice accessions used and their survival ability

S.N.	Accession	Survival (%)	S.N.	Accession	Survival (%)	S.N.	Accession	Survival (%)	S.N.	Accession	Survival (%)
1	Ujaraka Khareha	50	66	Silhaat	95	131	Sathaseto	10	196	NPGR 3121	0
2	Nimoe	80	67	Magar aanadi	30	132	Mahajogani	10	197	NPGR 3122	80
3	Anjana	90	68	Ratagola-1	40	133	Sothiyari	60	198	NPGR 3124	70
4	Rango	95	69	Jhinuwa	40	134	Chyaangnya	90	199	NPGR 3127	70
5	Mutmur	95	70	Bakulle Anadi	40	135	Rudrakshya	50	200	NPGR 3129	50
6	Wardhan	90	71	Kalonuniya	30	136	Diruwa	10	201	NPGR 3131	30
7	Ujaraka Basmati	45	72	Gahuma	40	137	FR13A	100	202	NPGR 3132	0
8	Ramjawaine	60	73	Jharlajhi	90	138	Goda Heenati	90	203	NPGR 3133	0
9	Harincave	60	74	Sikichan	60	139	Kurkaruppan	90	204	NPGR 3134	0
10	Annadi	50	75	Bagari-2	10	140	Nipponbarre	5	205	NPGR 3135	50
11	Ujaraka Jesariya	70	76	Ratoanadi	10	141	NPGR 2546	50	206	NPGR 3136	80
12	Anjana	75	77	Gokhalchan	60	142	NPGR 2555	50	207	NPGR 3137	50
13	Keshav Bachhi	10	78	Parewapakha	50	143	NPGR 2557	50	208	NPGR 3138	80
14	Mutmud	90	79	Dalle	50	144	NPGR 2558	40	209	NPGR 3139	100
15	Channanchud	60	80	Pakhal	90	145	NPGR 2561	40	210	NPGR 3141	100
16	Belasaro	90	81	Gorra	90	146	NPGR 2562	50	211	NPGR 3142	70
17	Gopal	60	82	Budidaiyan-3	50	147	NPGR 2568	70	212	NPGR 3175	75
18	Kalo saathi	40	83	Dudhraj	0	148	NPGR 2570	50	213	NPGR 7319	90
19	Sotwa	80	84	Thapachinni	0	149	NPGR 2573	70	214	NPGR 7364	50
20	Bagadi K	70	85	Khaaj	5	150	NPGR 2577	20	215	NPGR 6884	70
21	Duthani Saro	60	86	Ramuni	20	151	NPGR 2578	60	216	NPGR 3144	40
22	Bagadi B	70	87	Devshar	30	152	NPGR 2586	80	217	NPGR 3145	80
23	Bangaliya	80	88	Sathhi	0	153	NPGR 2587	80	218	NPGR 3148	100
24	Kalo Tulsi	40	89	Lalka Basmati	10	154	NPGR 2588	40	219	NPGR 3150	100
25	Pakhar	20	90	Kanhaar	20	155	NPGR 2590	60	220	NPGR 3154	100
26	Najir	60	91	Sotwa	10	156	NPGR 2591	20	221	NPGR 3155	100
27	Rajalla	70	92	Kankirabi	20	157	NPGR 3016	40	222	NPGR 3157	80
28	Bagari	60	93	Nakhisaro	20	158	NPGR 3018	40	223	NPGR 3209	60
29	Nyauri	10	94	Madhumala	20	159	NPGR 3019	40	224	NPGR 3211	50
30	Dhunmuniya Seto	50	95	Lajhi	90	160	NPGR 3020	40	225	NPGR 3212	100
31	Satthi-1	10	96	Badhudayan-2	90	161	NPGR 3036	70	226	NPGR 3227	60
32	Champa Saro	10	97	Jagranthiya	90	162	NPGR 3037	40	227	NPGR 3228	0
33	Seto Satthi	10	98	Brahambhushi	90	163	NPGR 3038	20	228	NPGR 3231	0
34	Nimoe-1	10	99	Guthani saro	90	164	NPGR 3039	40	229	NPGR 3267	10
35	Malaakheattae	10	100	Bhelasaro	90	165	NPGR 3040	10	230	NPGR 3270	10
36	Raimanuwa	90	101	Karangi	50	166	NPGR 3044	5	231	NPGR 3273	60
37	Seto Dalle	0	102	Sathokalo	30	167	NPGR 3049	5	232	NPGR 3274	50
38	Dhusara	60	103	Rango	60	168	NPGR 3050	50	233	NPGR 3275	100
39	Rameli Dhan	0	104	Aamaghauj	60	169	NPGR 3051	95	234	NPGR 3277	100
40	Karangi	90	105	Simthari	70	170	NPGR 3052	100	235	NPGR 3278	100
41	Jhapa Basmati	50	106	Kalodhan	50	171	NPGR 3053	100	236	NPGR 3281	100
42	Aanga	40	107	Cheudae	50	172	NPGR 3054	10	237	NPGR 3289	80
43	Lalka Jesariya	60	108	Gajargaul	90	173	NPGR 3055	15	238	NPGR 3290	80
44	Tunde Basmati	70	109	Setodalle	0	174	NPGR 3056	60	239	NPGR 3297	100
45	Batthi	90	110	Gheupuri	80	175	NPGR 3057	70	240	NPGR 3300	100
46	Nirmoe	90	111	Karangi	90	176	NPGR 3058	100	241	NPGR 3306	100
47	Gheukumari	40	112	Kataush	80	177	NPGR 3060	60	242	NPGR 3308	100
48	Ratagola-2	10	113	Kusumkaali	0	178	NPGR 3100	70	243	NPGR 3313	40
49	Nakkhisaro	30	114	Maalbhog	0	179	NPGR 3101	70	244	NPGR 3344	70
50	Bahaani	30	115	Chatraaj	10	180	NPGR 3102	0	245	NPGR 3345	60
51	Kasturi	40	116	Mansara	10	181	NPGR 3103	70	246	NPGR 3348	70
52	Rato tuppe	30	117	Ratin	10	182	NPGR 3104	60	247	NPGR 3359	0
53	Aamjhutte	30	118	Mala	50	183	NPGR 3105	50	248	NPGR 3362	0
54	Sakhar	80	119	Batsar	90	184	NPGR 3106	50	249	NPGR 3363	0
55	Mansara	80	120	Kariyakamod	50	185	NPGR 3107	80	250	NPGR 3469	0
56	Manamuri	80	121	Balmasaar	50	186	NPGR 3110	40	251	NPGR 3471	6
57	Sugapankhi	70	122	Bhaatsaar	95	187	NPGR 3111	60	252	NPGR 3487	52
58	Kalo tawa	50	123	Badhudaiyan	20	188	NPGR 3112	50	253	NPGR 3488	20
59	Lalchand	90	124	Dunmuniya seto	0	189	NPGR 3113	50	254	NPGR 3497	40
60	Ratanpuri	20	125	Jungali Mansuli	5	190	NPGR 3114	50	255	NPGR 3499	50
61	Localbuche	10	126	Dudhisaro	50	191	NPGR 3115	60	256	NPGR 3501	10
62	Pakhe jhinuwa	50	127	Jagad	90	192	NPGR 3116	650	257	NPGR 3502	70
63	Dudhisaro	70	128	Kataush	90	193	NPGR 3117	60	258	NPGR 3503	70
64	Sauthari	90	129	Kariyaparewa pakha	90	194	NPGR 3118	50	259	NPGR 3504	65
65	Laltanger	80	130	Kariyakheraha	40	195	NPGR 3119	80	260	NPGR 3508	40

Table 1: Continued

S.N.	Accession	Survival (%)	S.N.	Accession	Survival (%)	S.N.	Accession	Survival (%)	S.N.	Accession	Survival (%)
261	NPGR 3975	20	276	NPGR 8303	20	291	NPGR 8518	10	306	NPGR 8557	50
262	NPGR 3978	25	277	NPGR 8305	25	292	NPGR 8524	20	307	NPGR 8558	60
263	NPGR 3979	40	278	NPGR 8317	20	293	NPGR 8529	40	308	NPGR 8559	40
264	NPGR 3982	40	279	NPGR 8324	50	294	NPGR 8530	45	309	NPGR 8567	45
265	NPGR 3986	50	280	NPGR 8325	60	295	NPGR 8534	40	310	NPGR 8568	50
266	NPGR 3987	60	281	NPGR 8327	40	296	NPGR 8535	60	311	NPGR 8570	55
267	NPGR 3989	60	282	NPGR 8335	50	297	NPGR 8544	20	312	NPGR 8571	70
268	NPGR 3990	65	283	NPGR 8338	20	298	NPGR 8545	30	313	NPGR 8576	75
269	NPGR 3991	45	284	NPGR 8505	20	299	NPGR 8546	40	314	NPGR 8807	50
270	NPGR 3992	45	285	NPGR 8506	15	300	NPGR 8547	70	315	NPGR 9499	55
271	NPGR 3993	45	286	NPGR 8508	20	301	NPGR 8551	55	316	NPGR 9755	70
272	NPGR 3994	20	287	NPGR 8509	30	302	NPGR 8552	65	317	NPGR 9764	75
273	NPGR 3995	20	288	NPGR 8511	20	303	NPGR 8553	70			
274	NPGR 3996	25	289	NPGR 8513	50	304	NPGR 8554	60			
275	NPGR 4004	60	290	NPGR 8516	60	305	NPGR 8556	95			

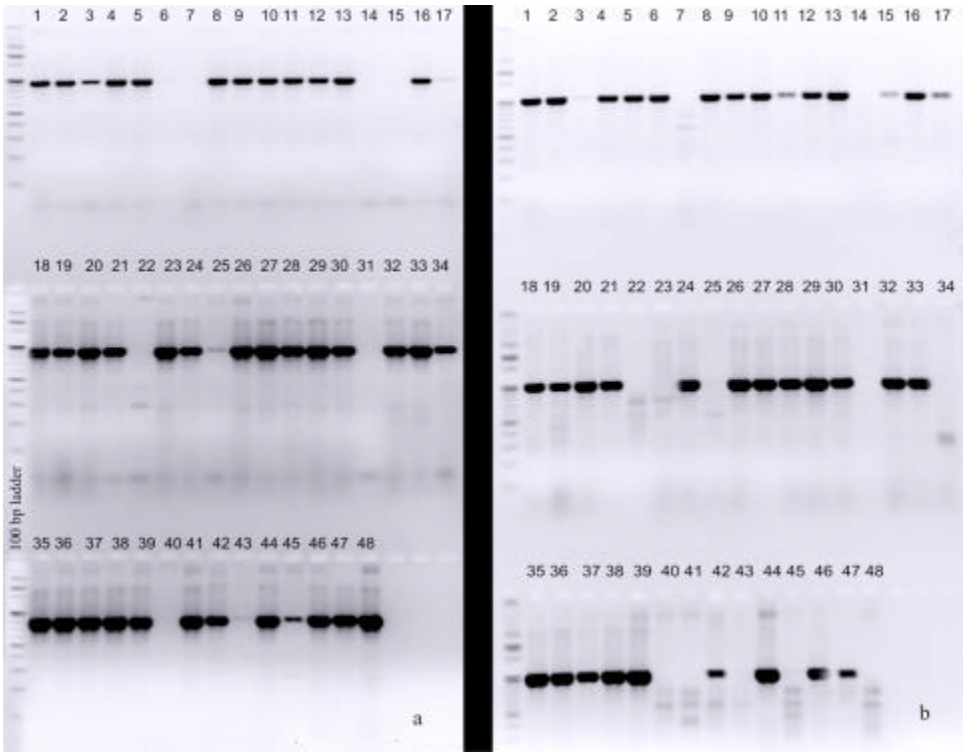

Fig. 3(a-b): Monitoring of *Sub1A* gene in 44 selected rice accessions. Six sets of *Sub1A* locus specific primer were used as reported earlier (Xu *et al*., 2006). (a) DNA amplification profiling to detect the presence and absence of *Sub1A* gene using *Sub1-A-7* primer couple, (b) Detection of *Sub1A-1* allele in tolerance and intolerance rice accessions using allele specific primer pair; *Sub1-A-1*. The detail information about the primer used are provided in the Table 3. Number in each lane represents the rice accessions as 1 = NPGR-3275, 2 = Goda Heenati, 3 = NPGR-3306, 4 = NPGR-3308, 5 = NPGR-3154, 6 = NPGR-3150, 7 = Nipponbarre, 8 = FR13A, 9 = NPGR-3148, 10 = NPGR-3277, 11 = NPGR-3200, 12 = NPGR-3297, 13 = NPGR-3139, 14 = NPGR-3278, 15 = NPGR-3212, 16 = NPGR-3281, 17 = Guthani Saro, 18 = Jharlajhi, 19 = Rango, 20 = Badhudayan-2, 21 = Mutmud, 22 = Anjana, 23 = Pakhal, 24 = Silhaat, 25 = Belasaro, 26 = Jagad, 27 = Kataush, 28 = Batsar, 29 = Brahambhushi, 30 = Mutmur, 31 = Lalchand, 32 = Jagranthiya, 33 = Wardhar, 34 = Gorra, 35 = Bhatti, 36 = Lajhi, 37 = Raimanuwa, 38 = Karangi=, 39 = Chyaangnya, 40 = Gajargaul , 41 = Kariyaparewa pakha 42 = Nirmoe, 43 = Sauthari, 44 = Bhaatsaar, 45 = Sikichand, 46 = Bhelasaro, 47 = Kurkaruppan and 48 = Bagadi-2

Table 2: Selected tolerant rice accession with their mean survival ability and status of *Sub1A* gene

Accession	*Sub1A*	*Sub1A-1*	Survival (%)	Accession	*Sub1A*	*Sub1A-1*	Survival (%)
Bhaatsaar	Present	Present	88.23	NPGR 3281	Present	Present	0
Kariyaparewa pakha	Present	Absent	85.08	Jharlajhi	Present	Present	0
Sauthari	Absent	Absent	79.44	Raimanuwa	Present	Present	0
Karangi	Present	Present	78.89	NPGR 3277	Present	Present	0
FR13A	Present	Present	71.67	NPGR 3148	Present	Present	0
Jagranthiya	Present	Present	66.67	NPGR 3275	Present	Present	0
Guthani Saro	Present	Present	63.47	Batsar	Present	Present	0
Kurkaruppan	Present	Absent	54.08	Nipponbarre	Absent	Absent	0
Rango	Present	Present	51.76	Bagadi-2	Present	Absent	ND
Lalchand	Absent	Absent	51.52	Belasaro	Absent	Absent	ND
Chyaangnya	Present	Present	48.93	Gorra	Present	Present	ND
Silhaat	Present	Present	41.28	Jagad	Present	Present	ND
Badhudayan-2	Present	Present	40.60	Kataush	Present	Present	ND
Goda Heenati	Present	Present	36.44	Mutmud	Present	Present	ND
Bhatti	Present	Present	34.89	Nirmoe	Present	Present	ND
Anjana	Absent	Absent	33.97	NPGI 3150	Present	Present	ND
Bhelasaro	Present	Present	28.41	NPGI 3154	Present	Present	ND
Mutmur	Present	Present	26.38	NPGR 3200	Present	Present	ND
Wardhar	Present	Present	20.43	NPGR 3212	Present	Present	ND
Lajhi	Present	Present	13.33	NPGR 3278	Present	Absent	ND
NPGR 3306	Present	Absent	8.89	NPGR 3297	Present	Present	ND
Gajargaul	Absent	Absent	4.78	NPGR 3308	Present	Present	ND
Brahambhushi	Present	Present	3.61	Pakhal	Present	Present	ND
NPGR 3139	Present	Present	0	Sikichand	Present	Absent	ND

ND: Not detected due to lack of enough number of rice seed to repeat the experiment

Table 3: List of primers used for *Sub1A* monitoring in selected Nepalese rice accessions

Primer name	Sequence (5'-3')	Tm (°C)	Cycles (n)	Size (bp)	Reference
*Sub1A*_1_fw	GATGTGTGGAGGAGAAGTGA	60	33	1015	Xu *et al.* (2006)
*Sub1A*_1_rev	GGTAGATGCCGAGAAGTGTA				
*Sub1A*_3_fw	CTCGGCACCTTCGACACC	56	33	685	Xu *et al.* (2006)
*Sub1A*_3_rev	AAGACGAACGGTGAACCATG				
*Sub1A*_4_fw	CTCGGCACCTTCGACACC	56	33	582	Xu *et al.* (2006)
*Sub1A*_4_rev	GGTAGATGCCGAGAAGTGTA				
*Sub1A*_5_fw	ATATTCACTGCTCACTAGTAAC	56	33	1040	Xu *et al.* (2006)
*Sub1A*_5_rev	GTTTGTGGCCTTTGAGTAAG				
*Sub1A*_6_fw	GATGTGTGGAGGAGAAGTGA	56	33	825	Xu *et al.* (2006)
*Sub1A*_6_rev	TGTTTTGGTGGATCGATGGG				
*Sub1A*_7_fw	GATGTGTGGAGGAGAAGTGA	56	33	932	Xu *et al.* (2006)
*Sub1A*_7_rev	GTTTGTGGCCTTTGAGTAAG				

Interestingly, eight rice accessions possessed the presence of *Sub1A-1* allele but did not tolerate 14 days of complete submergence (Table 2, Fig. 2a). In this experiment, though accessions; NPGR-3148, -3139 displayed the presence of *Sub1A-1*allele, they followed the escape adaptive strategy resulting the very poor survival ability under flash flooding (Fig. 2b). The chlorophyll retention after submergence was highest in FR13A than the tolerant accessions identified in this study. Unlike FR13A all the tolerant and intolerant rice accessions displayed the bleaching of chlorophyll pigment resulting the lower SPAD reading (Fig. 2c).

DISCUSSION

Flash flooding tolerance response in rice genotypes that have been evaluated so far harbor *Sub1A-1* allele of *Sub1A* locus and follow an energy saving quiescence growth mechanism (Xu *et al*., 2006; Bailey-Serres and Voesenek, 2008; Singh *et al*., 2010). Rice accessions with haplotypes other than *Sub1A-1/Sub1C-1* are intolerant to submergence (Singh *et al*., 2010). Similarly, deepwater response in rice and hypoxia tolerance in *Arabidopsis* are controlled by *SK1* and *SK2* and *RAP2.12, HRE1* and *HRE2* belonging to different members of subgroup VII of the ERF transcription factor family, respectively (Hattori *et al*., 2009; Licausi *et al*., 2011). Despite these earlier findings (Fukao *et al*., 2006; Xu *et al*., 2006; Fukao *et al*., 2011), in the present study some of the rice accessions such as Kariyaparewa pakha and Sauthari lacking *Sub1A-1* allele displayed the significant level of tolerance. Interestingly, contrasting response was also observed for rice accessions namely NPGR 3139, NPGR 3281, Jharlajhi, Raimanuwa, NPGR 3277, NPGR 3148, NPGR 3275 and Batsar. These rice accessions contained *Sub1A-1* allele but the recovery rate was close to nil (Table 2, Fig. 3b). The poor survival ability in those genotypes could be explained by extra costing of energy for rapid

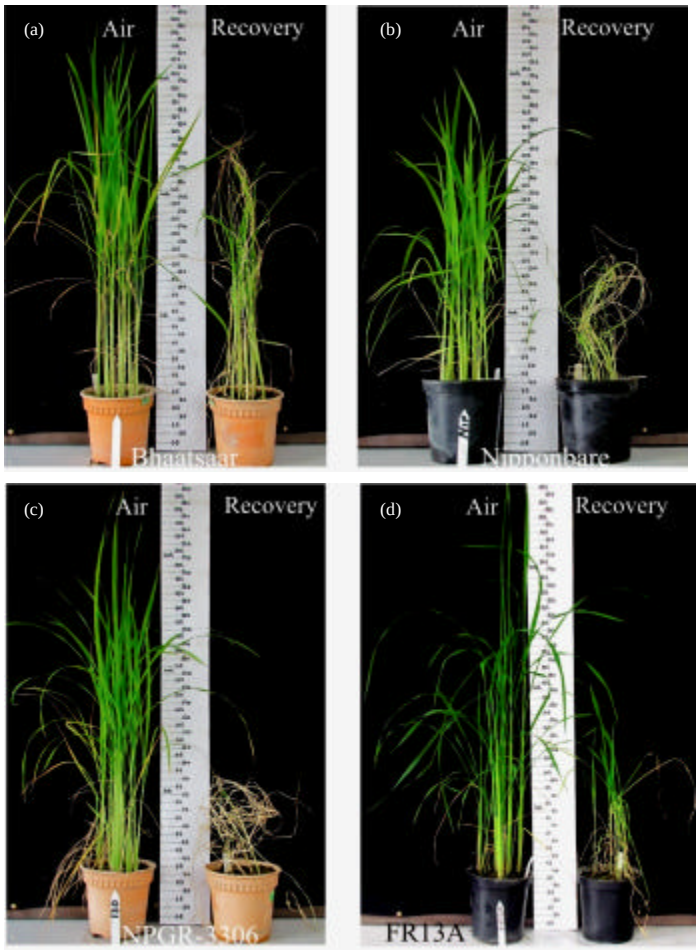

Fig. 4(a-d): Representative photographs showing 7 d of recovery after 14 d of complete submergence in selected rice accessions

stem elongation response (Fig. 2b) which is to be considered disadvantage for faster regeneration (Perata and Voesenek, 2007; Colmer and Voesenek, 2009). Underwater elongation in rice is triggered by ethylene and GA (Fukao *et al*., 2006). It is suggested that, in the absence of *Sub1A-1*, *Sub1C* facilitates shoot elongation during drowning, through a GA-dependent mechanism (Fukao and Bailey-Serres, 2008). *Sub1A-1* reverses the ethylene-dependent increase in GA responsiveness and consequent *Sub1C* mRNA accumulation. However, based on recombinant genetic studies, *Sub1A* seems to be the major determinant of submergence tolerance, as *Sub1C* gene expression does not significantly affect the level of tolerance (Septiningsih *et al*., 2009).

This study also clearly indicated that the chlorophyll retention capacity after submergence varied with the overall genetic make up rather than the presence of *Sub1A-1* allele. FR13A was found to be robust interms of chlorophyll retention capacity and the slow depletion mechanism of chlorophyll could be plus that helps to replenish the energy crisis through instant photosynthesis during recovery (Fig. 2c, 4). Positive correlation between survival and chlorophyll content after submergence was also reported by Das *et al*. (2009). However, most tolerant rice accessions identified in this study such as Bhaatsaar, Karangi and Sauthari possessed rapid depletion of chlorophyll signifying that chlorophyll retention capacity of the genotypes is independent of submergence tolerance trait. Compare to tolerant check, four genotypes viz. Bhaatsaar, Kariyaparewa pakha, Sauthari and Karangi performed better however, the mechanism of regeneration was quite dissimilar (Fig. 4). In these accessions most of the older leaves died soon after the desubmergence but regeneration of new leaves was

abruptly rapid (Fig. 4 and Table 2). Unlike these accessions, upon desubmergence the older leaves of FR13A showed low level of senescent and regained its normal growth very soon.

Based on submergence response and monitoring of *Sub1A* gene among the 28 rice accessions we hypothesized that in the absence of gene regulating elongation response, the survival ability is independent of *Sub1A-1*. On the other hand, in the presence of gene responsible for energy consumption process and absence of *Sub1* haplotype (*Sub1A-1/Sub1C-1*), *Sub1A-1* allelic form alone is not sufficient to confer the submergence tolerance. To validate these hypotheses *Sub1* haplotypic analysis, transcriptomic and hormonal profilings are underway.

CONCLUSION

Following large scale screening of 313 diverse rice accessions we are able to identify four tolerant genotypes. These genotypes were with and without *Sub1A-1* allele. From the perspective of rice improvement these germplasm constitute the great assets to broaden the narrow genetic base of submergence tolerance trait. Despite their potential importance, the exact mechanisms of tolerance in those accession are to be unraveled. Therefore, to dissect the underlying hidden mechanisms, further in-depth study using a combined developmental, physiological and omics approaches are suggested.

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REFERENCES

Bailey-Serres, J. and L.A. Voesenek, 2008. Flooding stress: Acclimations and genetic diversity. Annu. Rev. Plant Biol., 59: 313-339.

Colmer, T.D., L.A.C.J. Voesenek, 2009. Flooding tolerance: Suites of plant traits in variable environments. Funct. Plant Biol., 36: 665-681.

Das, K.K., D. Panda, R.K. Sarkar, J.N. Reddy and A.M. Ismail, 2009. Submergence tolerance in relation to variable floodwater conditions in rice. Environ. Exp. Bot., 66: 425-434.

Fukao, T., K. Xu, P.C. Ronald and J. Bailey-Serres, 2006. A variable cluster of ethylene response factor-like genes regulates metabolic and developmental acclimation responses to submergence in rice. Plant Cell, 18: 2021-2034.

Fukao, K. and J. Bailey-Serres, 2008. Submergence tolerance conferred by *Sub1A* is mediated by *SLR1* and *SLRL1* restriction of gibberellin responses in rice. Proc. Natl. Acad. Sci., 105: 16814-16819.

Fukao, T., E. Yeung and J. Bailey-Serres, 2011. The submergence tolerance regulator *SUB1A* mediates crosstalk between submergence and drought tolerance in rice. Plant Cell, 23: 412-427.

Hattori, Y., K. Nagai, S. Furukawa, X.J. Song and R. Kawano *et al*., 2009. The ethylene response factors *SNORKEL1* and *SNORKEL2* allow rice to adapt to deep water. Nature, 460: 1026-1030.

Hossain, M. and M.Z. Abedin, 2004. Rice research and development in the flood-prone ecosystem: an overview. Proceedings of the international workshop on flood-prone rice systems, January 9-11, 2001, Gazipur, Bangladesh, pp: 1-10.

Jung, K.H., Y.S. Seo, H. Walia, P. Cao and T. Fukao *et al*., 2010. The submergence tolerance regulator Sub1A mediates stress-responsive expression of AP2/ERF transcription factors. Plant Physiol., 152: 1674-1692.

Khush, G.S., 1997. Origin, dispersal, cultivation and variation of rice. Plant Mol. Biol., 35: 25-34.

Lee, K.W., P.W. Chen, C.A. Lu, S. Chen, T.H. Ho and S.M. Yu, 2009. Coordinated responses to oxygen and sugar deficiency allow rice seedlings to tolerate flooding. Sci. Signal., Vol. 2, 10.1126/scisignal. 2000333.

Licausi, F. and P. Perata, 2009. Low oxygen signaling and tolerance in plants Adv. Bot. Res., 50: 140-198.

Licausi, F., M. Kosmacz, D.A. Weits, B. Giuntoli, F.M. Giorgi, L.A.C.J. Voesenek, P. Perata and J.T. van Dongen, 2011. Oxygen sensing in plants is mediated by an N-end rule pathway for protein destabilization. Nature, 10.1038/nature10536.

Mackill, D.J., W.R. Coffman and D.P. Garrity, 1996. Rainfed Lowland Rice Improvement. International Rice Research Intitute, Manila, ISBN-971-22-0071-X,.

Nagai, K., Y. Hattori and M. Ashikari, 2010. Stunt or elongate? Two opposite strategies by which rice adapts to floods. J. Plant Res., 123: 303-309.

Perata, P. and L.A. Voesenek, 2007. Submergence tolerance in rice requires *Sub1A*, an ethylene-response-factor-like gene. Trends Plant Sci., 12: 43-46.

Septiningsih, E.M., A.M. Pamplona, D.L. Sanchez, R. Maghirang-Rodriguez and C.N. Neeraja *et al*., 2009. Development of submergence-tolerant rice cultivars: The *Sub1* gene and beyond. Ann. Bot., 103: 151-160.

Singh, N., T.T. M. Dang, G.V. Vergara, D.M. Pandey and D. Sanchez *et al*., 2010. Molecular marker survey and expression analyses of the rice submergence-tolerance gene *Sub1A*. Theor. Appl. Genet., 121: 1441-1453.

Sul, I.W. and S.S. Korban, 1996. A highly efficient method for isolating genomic DNA from plant tissues. Plant Tiss. Cult. Biotech. 2: 113-116.

Voesenek, L.A.C.J. and J. Bailey-Serres, 2009. Genetics of high-rise rice. Nature, 959: 960-10.1038/460959a.

Xu, K., X. Xu, T. Fukao, P. Canlas and R. Maghirang-Rodriguez *et al*., 2006. *Sub1A* is an ethylene-response-factor-like gene that confers submergence tolerance to rice. Nature, 442: 705-708.