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Expression of stress related genes associated with CDPK, ABA-dependent and -independent signaling pathways in highly submergence tolerant rice germplasm

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Abstract

In submergence tolerance screening of 111 rice germplasms, we noted the survival rate above 70 percent in thirteen rice germplasms. Among them, only one germplasm, *Dhala putia* showed the highest survival rate (95%) compared to tolerant genotype, FR13A (70%). In PCR, Sub1 QTL encompassing SUB1A, SUB1B and SUB1C genes were confirmed in *Dhala putia* using gene-specific DNA markers. In gene expression analysis, induces of expression of OsDREB1A, OsDREB2A and OsCDPK7 genes related to Abscisic acid (ABA)-dependent,- independent pathways and Ca^{2+}-dependent protein kinase (CDPK) signaling pathways in *Dhala putia* genotype was documented under various abiotic stress conditions compared to FR13A.

Key words : DREB1A, OsCDPK7, rice landraces, abiotic factors, gene expression.

Introduction

Submergence of rice (*Oryza sativa* L.) during the monsoon ûooding season seriously limits rice production and it causes approximate annual loss of over one billion U.S. dollars in south and southeast Asia. In rainfed lowland cultivation areas, most rice cultivars subject to death due to complete submergence for 1 to 2 weeks. To overcome this problem, a major locus, Submergence1 (Sub1) on chromosome 9 has been identified for submergence tolerance and so far, many rice cultivars are/being improved using this major Sub1 QTL worldwide **(Septiningsih et al. 2009**). Meanwhile, the global climate change further aggravate the situation to more extreme weather events. Therefore, further enhancement in tolerance or combining more than one trait is necessitated according to recent surveys in countries affected by extreme weather**.** In this study, 111 rice germplasms **(Table 1)** were screend for the higher survival rate under submergence condition and its tolerant mechanism was studied at molecular level. For submergence screening, 111 rice germplasms at 21-days old seedling stage were submerged into water tank for 2-weeks in presence of tolerant genotype FR13A and intolerant check, IR42 **(Septiningsih et al. 2009).** In this screening, the survival rate was noted in the range of 0-95 per cent among these germplasms (Table 1) and this variation in the survival rate is associated with very slow (tolerant) and fast (intolerant) process of starch consumption and soluble sugars to maintain growth elongation in rice, respectively. In this screening, 13 rice germplasms i.e. *Mugei* (80%), *Kalaketaki* (80%), *Dhala putia* (95%), IC253330 (85%), AC20431B (70%), IC258990 (84%), EC 516602(75%), AC37887 (85%), *Kusuma* (75%), *Gangasiuli* (70%),

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Longalamunda (84%), *Pohasali* (70%), *Bodala Champa* (78%) showed more than 70 per cent survival rate and they were considered as submergence tolerants. However, among them, only one germplasm, Dhala putia accounted for the highest survival rate (95%) compared to FR13A (70%)(Fig.1). In order to gain insight, *Dhala putia* genotype was confirmed by matching banding pattern of FR13A using gene-specific markers (IYT1, Sub1A203, Sub1BC2, ART5 and Sub1C173 markers) **(Septiningsih et al. 2009)**(Fig.2). Furthermore, *Dhala putia* genotype was subjected to different abiotic stresses as follows: submerging into water (submergence), withholding water irrigation (drought), dipping in nutrient solution containing 250mM NaCl (salt), keeping at 4ºC (cold) and 37ºC (heat) for 24 h in presence of FR13A and gene expression of OsDREB1A, OsDREB2A and OsCDPK7 was analysed through reverser transcriptase (RT)-PCR (**Fukao et al. 2011; Dubouzet et al. 2003; Saijo et al. 2000).** The primer sequence of Actin1 was used as loading control. Total RNA from leaves of *Dhala putia* and FR13A seedlings under stress and normal condition was extracted using TRIzol according to manufacturer's instructions. Then, cDNA was synthesized according to manufacturer's instructions (Invitrogen, California, USA) in a reaction mixture containing 50–75 ng RNA for 20µL at 50ºC for 1h. In RT-PCR analysis, significant differences in expression of OsDREB1, OsDREB2A and OsCDPK7 genes between *Dhala putia* and FR13A under salt, cold, drought, heat and submergence stress conditions was detected (Fig.3). OsCDPK7 gene was expressed in response to cold and submergence in *Dhala putia* but not induced in FR13A. Induce of OsCDPK7 gene expression in *Dhala putia* genotype under cold and submergence conditions indicate the involvement of Ca^{2+} -dependent protein kinase (CDPK) pathway. Expression of OsDREB1A gene was induced strongly in response to cold and slightly in salt in *Dhala putia*, but in FR13A, its strong expression was found in salt and heat stress. Expression of OsDREB2A gene was found to be induced strongly in *Dhala putia* under cold, salt, drought and submergence stress conditions. In FR13A, OsDREB2A gene expression was detected only in submergence and cold stresses. In *Dhala putia* genotype, induces of OsDREB2A gene expression in response to cold and submergence stress indicates the cross-talk between DREB1A and DREB2A transcript factors. Because the expression of DREB2A is induced by drought/salt/ heat stress but not by cold **(Liu et al. 1998)**. And, Sub1A, Sub1B and Sub1C genes in Sub1 locus are identified as putative ethylene-responsive transcription factor (ERF) belonged to the APETALA 2/ethylene-responsive element binding factor (AP2/ERF) family **(Xu et al. 2006)**. In a study, **Fukao et al. (2011)** have reported the accumulation of DREB1A and DREB1E mRNA in rice line with Sub1QTL under drought stress compared to rice line with no SUB1. Thus, in these two genotypes, transcript factors linked with ABA-dependent and –independent pathways involve in tolerance to various abiotic stresses by cross-talk. Additionally, expression of OsCDPK7 gene detected in *Dhala putia* genotype under cold and submergence conditions indicates the association of CDPK signaling pathway also in stress tolerance alongwith ABA-dependent and –independent pathways. According to **Sanders et al. (1999)** the level of cytoplasmic Ca^{2+} in plant cells is increased in response to cold, salt and drought rapidly and the signal is mediated by members of the CDPK family in plants to induce Abscisic acid (ABA) and stress-responsive gene transcription. Perhaps, in this study, the expression of OsCDPK7 might have associated with the higher survival rate in *Dhala putia* genotype during submergence stress by inducing cold genes through CDPK signaling pathway compared to FR13A. Submergence challenge is conceivably accompanied by low-temperature stress as flood water temperatures are likely lower than ambient air temperatures **(Jung et al. 2010)**. This study has revealed the variations in the mode of signaling pathways from genotype to genotype as well as the highest tolerance in genotype with more number of signaling pathways. Thus, the transcription factors

(TFs), by cross-talking with each other, have likely to regulate the developmental, physiological and biochemical responses of plants to a variety of environmental stress conditions **(Mizoi et al. 2012).** Therefore, understanding the mechanisms by which plants transmit the signals to cellular machinery to activate adaptive responses is of fundamental importance to develop more stress tolerant crops to improve production efficiency.

Acknowledgements

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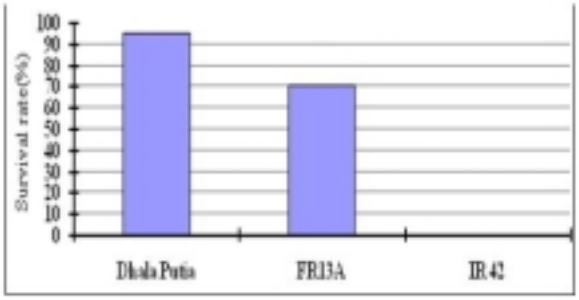

Figure 1. Survival rate of *Dhala putia*, FR13A landraces and IR42 (Intolerant check) followed by submergence stress for 2-weeks.

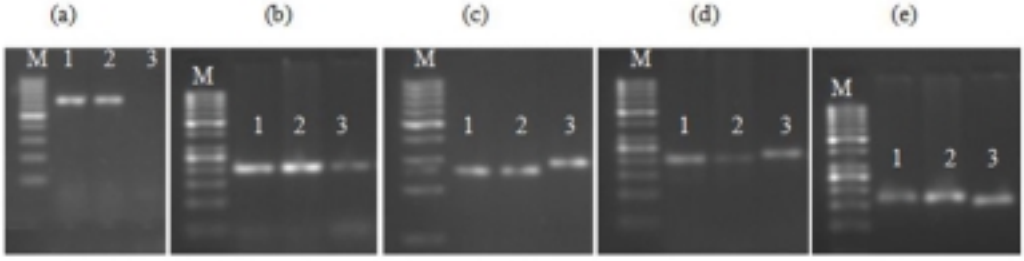

Figure 2. PCR results of *Dhala putia*, FR13A landraces and IR42 (Intolerant check) with gene-specific markers located within Sub1QTL. (a):IYT1 (promoter region of Sub1A gene); (b):Sub1A203 (exon of Sub1A); (c):Sub1BC2 (region between Sub1B and Sub1C gene); (d):ART5 (promoter region of Sub1C gene); (e):Sub1C173 (exon of Sub1c gene).

Lane1:FR13A; 2:*Dhala putia*; 3:IR42. M-100bp DNA ladder.

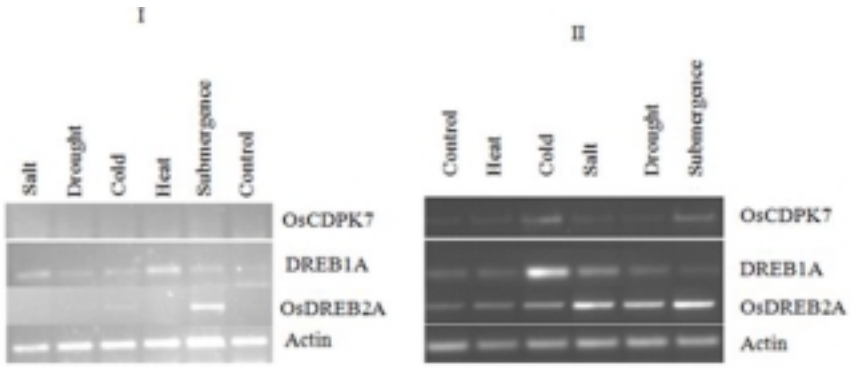

Figure 3. Reverse transcriptase (RT)-PCR analysis.

Expression of stress-inducible genes in 14-days-old seedlings of FR13A (I) and *Dhala putia* (II) landraces under salt (250mM NaCl), drought (no water), cold (4°C), heat (37°C) and submergence stress conditions at 24 h.

Table 1.[1]. Details of 111 rice germplasms and their survival rate in submergence screening

S.No.	Genotype	Survival rate (%)	S.No.	Genotype	Survival rate (%)
1.	**Mugei**	**80.0**	58.	Chengul	0.0
2.	**Kalaketaki**	**80.0**	59.	No.56(white kernel)	0.0
3.	Asina	58.0	60.	Kaliroy	30.0
4.	Hirakani	0.0	61.	Chenun	5.0
5.	Champasula	40.0	62.	Kala Chengul	25.0
6.	Putia	21.0	63.	Baikoili	20.0
7.	Khadiasula	0.0	**64.**	**Dhala Putia**	**95.0**
8.	Banusagaja	30.0	65.	Chaka akhi (1590)	10.0
9.	Bhundi	45.0	66.	Orihan	47.0
10.	Aksani	20.0	67.	Kedargauri	10.0
11.	Gitanjali	12.0	68.	Mayurkantha	12.0
12.	Dudhesar	20.0	69.	Gudmatia	17.0
13.	Suna Mugdi	0.0	70.	Panchayat Pateli	25.0
14.	Udisiali	35.0	71.	Nali khuda	10.0
15.	Nausal	0.0	72.	Kartika rangi	0.0

Table 1.[1]. Details of 111 rice germplasms and their survival rate in submergence screening

S.No.	Genotype	Survival rate (%)	S.No.	Genotype	Survival rate (%)
16.	Rebena	0.0	73.	Kalamula	15.0
17.	Betna	30.0	74.	Betanatia	0.0
18.	Kalakandhi	35.0	75.	Malabati	20.0
19.	Dusali	0.0	76.	Kedar Gauri	12.0
20.	Kalabhuta	10.0	77.	Nidhia	11.0
21.	Khejurchhadi	20.0	78.	Baula	0.0
22.	Sagra(Malabati)	35.0	79.	Matia	45.0
23.	Janglijhata	10.0	80.	Rahaspanjar	30.0
24.	Dangar Patnai	28.0	81.	Godi Khadara	25.0
25.	Lal Patnai	10.0	82.	Banki kalam	28.0
26.	Langal Munda	40.0	83.	Pooja	0.0
27.	Lalchamori	25.0	84.	Durga	20.0
28.	Swet Chamori	0.0	85.	Gangasuili	0.0
29.	Sabitar cross-white	20.0	86.	Biothal Pakhia	29.0
30.	Sabitar cross-brown	5.0	87.	Ravana	22.0
31.	Madhyaraj	0.0	88.	Matiaburusu	45.0
32.	Lakhigajar	0.0	89.	Makhara	20.0
33.	Agniban	10.0	90.	AC20431W	30.0
34.	Paijam	10.0	**91.**	**IC 253330**	**85.0**
35.	Kala Nunia/Bhog D	0.0	**92.**	**AC 20431B**	**70.0**
36.	Bajal	0.0	**93.**	**IC 258990**	**84.0**
37.	Sagarica	10.0	**94.**	**EC 516602**	**75.0**
38.	Benasali	0.0	**95.**	**AC 37887**	**85.0**
39.	BM-6	15.0	96.	AC 38575/37570	55.0
40.	Naora	0.0	**97.**	**Kusuma**	**75.0**
41.	Dhyapa	5.0	98.	Kalaputia	0.0
42.	Haijam	0.0	**99.**	**Gangasiuli**	**70.0**
43.	Gangia	25.0	100.	Atiranga	40.0
44.	Haldijam	0.0	101.	Matiaburusu	30.0
45.	Jadudhan	0.0	102.	Gayatri	0.0

Table 1.[2] . Details of 111 rice germplasms and their survival rate in submergence screening

S.No.	Genotype	Survival rate (%)	S.No.	Genotype	Survival rate (%)
46.	Maheswari	0.0	103.	Sarala	0.0
47.	Dodh Kalam/Hemti	20.0	104.	Varshadhan	40.0
48.	Beto	20.0	105.	Balia Dhaka	20.0
49.	Dodh Kalam	25.0	**106.**	**Longalamunda**	**84.0**
50.	Chengul	33.0	**107.**	**Pohasali**	**70.0**
51.	Kamat	0.0	**108.**	**Bodala Champa**	**78.0**
52.	Chota Bhusa	0.0	109.	Jangala Jhata	5.0
53.	Bansmulsari	0.0	110.	Khoda	65.0
54.	Pansira	30.0	111.	Hatimala	55.0
55.	Pandhali	0.0	112.	**FR 13A(Tolerant)**	**70.0**
56.	Bahorva	15.0	113.	IR42(Intolerant check)	0.0
57.	Utpal	5.0			

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