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IDENTIFICATION OF RICE SPECIFIC DROUGHT RESPONSIVE CANDIDATE GENES IN ABA INDEPENDENT PATHWAY FROM TOLERANT AND SUSCEPTIBLE VARIETIES OF *ORYZA SATIVA* L.

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ABSTRACT

For two-thirds of the world's population, rice, the second-most-cultivated cereal, is essential. The creation of drought-tolerant cultivars is necessary since crop quality and productivity are impacted by drought stress. On 24 rice types, a study examined 14 potential genes from the ABA independent pathway. The findings demonstrated that genes relevant to drought were present in tolerant species clones and present in vulnerable accessions. This suggests a complicated system involving numerous genes operating in various pathways to determine whether a genotype is resistant to or tolerant of drought. This strategy aids in boosting food availability and satisfying rising worldwide demand. Rice, in present study, 14 putative candidate genes belonging to abscisic acid (ABA) independent pathway were screened on 24 different rice (*Oryza sativa* L.) varieties. The existence of a few drought responsive genes in susceptible accessions of *O. sativa* and absence of some drought specific genes in tolerant species clones were noticed. This observation leads to the existence of complex machinery involving several genes in multiple pathways that finally makes a genotype susceptible or tolerant to drought.

Key words : Candidate gene, Rice, Drought, Abscisic Acid, ABA-Independent Pathway.

Introduction

Rice (*Oryza sativa* L.) is a major and staple foodcrop in many parts of the world, feeding more than three billion people and providing 50-80% of their daily calories intake (Khush, 2005). Rice grows in the tropics, subtropics, semi-aridtropics and temperate regions of the world. More than 90% of the world's rice is produce in Asia (Roy and Misra, 2002; Zhai *et al*., 2023). It is a drought susceptible crop exhibiting serious deleterious effects, when exposed to water stress at critical growth stages especially at reproductive stage (Suriyan *et al*., 2010). Drought is one of the major abiotic stresses that severely affect and reduce the yield and productivity of food crops worldwide up to 70% (Kaur *et al*., 2008; Akram *et al*., 2013). Breeding for drought tolerance is a challenging task because of the complexicity of the component traits, screening technique, environmental factors and their interaction. Drought triggers production of the phytohormone abscisic acid (ABA), which in turn causes stomatal closure and induces expression of stress-related genes. Several drought-inducible genes are induced by exogenous ABA treatment, whereas, others are not affected. Indeed, evidence exists demonstrating the presence of both ABA-independent and ABA-dependent regulatory systems governing drought-inducible gene expression (Liu *et al*., 1998; Yamaguchi-Shinozaki and Shinozaki, 2005). Identification of genetic factors involved in plant responses to drought stress will provide a solid foundation to breed plants with improved drought tolerance. In this regard, candidate genes are useful, and their identification starting with selection of some target genes based on the biochemical pathway could pave the way for developing an effective breeding approach for drought tolerance (Sanchez *et al*., 2002). A number of genes have been reported to be induced by drought, high salinity, and low-temperature stresses and their products

are thought to function in stress tolerance response (Bray, 1997; Shinozaki and Shinozaki, 2000). ABA-dependent and ABA-independent pathways lead to rapid responses to drought or cold and function through members of the AP2/ERF family of transcription factors (Shinozaki and Shinozaki, 1994). In this study, ABA independent genes identified in other plant species were tested for their role in drought tolerance in rice, based on the analysis on a set of drought tolerant and susceptible varieties.

Materials and Methods

The plant materials were obtained from the Department of Genetics and Plant Breeding, Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad, Uttar Pradesh, India. Table 1

Table 1 : List of rice varieties belonging to *Oryza sativa* L. species.

S. no.	Name of variety	Parentage	Year of notification	Duration (in days)	Eco- System	Recommended for cultivation
1.	NDR-97	N-22 x Ratna	1992	90-95	Rain fed Uplands	Uttar Pradesh, Orissa and West Bengal
2.	Pusa Basmati-1	Pusa-150 × Karnal Local	1989	135	Irrigated Areas	Goa, Mizoram and Uttar Pradesh
3.	NDR-118	IR-36 × Hansraj-A	1988	85-90	Rainfed Uplands	All India
4.	NDR-359	BG-90-2-4 × 08677	1994	115-125	Irrigated Areas	Uttar Pradesh, Bihar and Orissa
5.	ShuskSamrat	C 1064-5/Kalari/ IR 54	2006	100-105 (Early)	Upland (Direct seeded), mod. drought tolerant	Bihar, Orissa and Uttar Pradesh
6.	NDR-1	Bella Patna × IR-8	1983	105	Irrigated or Rainfed	Uttar Pradesh.
7.	Nagina-22	A selection from Rajbhog	1978	85-102	NA	Uttar Pradesh.
8.	KUHUSOI-RI-SAREKU	IRRI lines	_	110-121	_	_
9.	IR 91167-31-3-1-33	IRRI lines	_	120-128	_	Eastern india
10.	R-RHZ-2	_	_	115-125	_	Eastern india
11.	IR 92960-75-1-3	IRRI lines	_	100-105	_	Eastern india
12.	IR 68144-2B-2-2-3-1-120	IRRI lines	_	125-130	_	_
13.	AMKER	_	_	108-119	_	Eastern india
14.	TARAMON	_	_	128-135	_	Eastern india
15.	IR 83668-35-2-2-2	IRRI lines	_	117-124	_	_
16.	AYAAR	_	_	156-165	_	_
17.	IR 68144-2B-2-2-3-1-127	IRRI lines	_	112-120	_	_
18.	NGOBANYO RED COVER	_	_	112-123	_	_
19.	IR 91167-133-1-1-2-3	IRRI lines	_	115-126	_	_
20.	MAIGOTHI	_	_	119-128	_	_
21.	Sarjoo-52	T(N)1 x Kashi	1982	130-133	Irrigated	Uttar Pradesh.
22.	Barani Deep	C 1064-5/IR 9129-320-3-3-3/IR 54	2006	95-100	Rainfed upland/ irrigated	Uttar Pradesh
23.	IR-64	IR-5857-33-2-1 × IR-2061-465-1-5-5	1991	115-120	Irrigated Areas	All India
24.	GOPALBHOK (LOCAL)	_		128-133	_	_

Table 2 : List of candidate genes belonging to ABA independent pathway.

S. no.	Pathway	Gene/ primer	Forward primer (5'- 3') Reverse primer (3'-5')	Gene included	Annealing temperature	Product size (bp)
1.	Drought responsive regulatory factors	DREB 1A	F-AGATGTGCGGGATCAAGCAG R-TCGCGTAGTACAGGTCCCAG	Dehydration response element binding factor 1A	56	500
		DRF 1	F-AGCAAGCTCAAGCAGTCAGT R-GGGGTTGGCTGTCAAGCTTA	Dehydration-responsive factor 1	56	200
		HRD	F-TGGCGGCAATAGCCTATGAC R-CTATTCATGCAAGCCACAC CAC	Hardy transcription factor	55	420
		NAC 2	F-GGTTGCTGGCCACCATTTCT R-GCCGTTTGGTACCTTCTGCT	NAC-like transcription factor 2	55	305
		POM1	F-CTGATCTGCATTGCGGCTTG R-CAGACCCCAAGCTAAAGGCC	Chitinase	56	800
		SHN 1	F-ATCCTCAGCGCCAAACTGAG R-GTGGTCGGAGCAAGAATAG CG	SHINE 1 Transcriptional factor	55.6	308
		SIZ 1	F-TGTAGCCAACGGCATGGAAC R-TCTCAGACAGGGAACAAAC CAG	Sumo ligase1	55	205
		Wrky 38	F-CGTGGTGTTTGAGGGACCAA R-GTACGTCGCCACGAGTATGG	Wrky transcription factor 38	56	500
		ZF1	F-ATCAAGTCGACGGTGGAGACT R-CCATGGGAAACTCCACTCCG	Zinc finger protein 1	57	270
2.	Transcription factor for salt tolerance	Snac1	F-TACAAGTTCGACCCGTGGGA R-GCGACGAGTAGAAGTCGCC	Stress responsive nac1	55.3	210
3.	Auxin dependent regulatory factors	NIT 1	F-ATCCCCGTTTACGACACT R-ACGAAACATCCACCTTC	*Nitrilase 1*	47	150
		PIN 3	F-ACGTTTTCGGCGGAGCACCG R-TGCCACTGAATTCCCACAAC	Polar auxin transport genes	58	180
4.	Enzymes encoding protective factors	Hep2	F-TGCCTGCCGTTGCATAGATG R-TTAGTGCCGTTGAATGTTGCC	Heparanase	54	550
		HYD3	F-TGAGCTGTAACGCTTGGAGGT R-GCCATGCCGAACAGCGTAAT	β-carotene hydroxylase	54	280

illustrates the list of 24 drought tolerant and susceptible varieties belonging to *Oryza sativa* L. species taken for the study.

Candidate genes

Candidate gene is a gene whose function suggested that it may be involved in the genetic variation observed for a particular trait. In order to find the putative drought responsive genes, the public database was exhaustively searched for gene sequences and/or regulatory sequences important for enhanced drought resistance along with an elaborate literature survey. The putative gene sequences were identified by reviewing published literature in several academic database search engines such as PubMed®, Web of Knowledge® and Google

search and the candidate gene searched from the Priji and Hemaprabha 2014.

Genomic DNA isolation and purification

Leaf samples were collected from the rice varieties given in Table 1 and DNA isolation and purification were carried out by CTAB method (Doyle and Doyle, 1987). The quality, quantity and purity of DNA were checked in Nano Drop ND-1000- DNA / RNA quantifier.

Designing the candidate genes

The nucleotide sequences of the genes of interest were identified by using the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/). The forward and reverse primers of the respective genes were designed using the Primer 3 software (http://frodo.wi.mit.edu/cgi bin/primer3/primer3_www.cgi) (Rozen and Skaletsky, 2000).

PCR amplification and electrophoresis for candidate gene evaluation

PCR reactions were performed in all the rice varieties using the primers of 14 drought tolerant genes in a MJ thermal cycler PT 100. Each PCR reaction was carried out with a total reaction volume of 10µl containing 25 ng of genomic DNA, 1X *Taq* buffer with $MgCl_2$, 100 µM dNTPs, 0.3 unit of *Taq* DNA polymerase enzyme and 20-30 pmol of each forward and reverse primer. The amplification conditions included an initial denaturation at 94^{0}C for five minutes, followed by 35 cycles of 94^{0}C for 30 seconds for denaturation, primer annealing at 58-62^{0}C (based on the Tm value of gene specific primers used for the study) for 30 seconds, 72^{o}C for 30 seconds for extension and a final extension at 72^{o}C for seven minutes. Upon completion of the PCR cycles, the amplified products were mixed with 2µl of loading dye (6X) and were resolved on 2% agarose gel (low EEO) containing ethidium bromide (0.2mg/ml) for 40 minutes at 100 W. The gels were observed using Alpha Imager Imaging system (Alpha Innotech) and drought specific bands were identified.

Fourteen candidate genes belonging to ABA independent pathway were selected to study drought response in rice. These genes included *DREB 1A*, *DRF1*, *Hep2*, *HRD*, *HYD3*, *NAC 2*, NIT 1,*PIN 3*, *POM1*, *SHN 1*, *S1Z 1*, *Snac1*, *Wrky 38* and *ZF1*. The genes studied fell into groups such as drought responsive regulatory factors, dehydration responsive element transcription factors for drought and salt stress, auxin mediated regulatory factors and genes encoding protective factors. Table 2 shows the list of fourteen genes associated with drought tolerance genes, forward and reverse primers and the pathway to which they belong.

Results and Discussion

The plant genes identification for drought tolerance is very important. Candidate gene analysis was used to identify drought specific genes in tolerant genotypes. In the present study, out of fourteen drought responsive genes independent of ABA pathway analyzed, thirteen genes were amplified in the different varieties, showing their specificality to drought tolerance in rice. While one candidate genes *Hep2* essential for a drought tolerant gene identified in rice (S. Subharmanyam) did not exhibit any amplification in the varieties. The gene can be amplified in different susceptible and tolerant variety is shown in Figs. 1A-1M.

Three candidate genes *viz.*, *DRF 1*, *SIZ 1* and *DREB1A* factor were uniformly present in most varieties but all three are not present in NDR-359; it is a highly drought susceptible variety. Both *DRF1* and *SIZ 1* not present in pusa basmati-1 and NDR-359. *HRD* - a drought and salt tolerance gene (Aarati *et al.*, 2007) showed presence of bands of 420 bp in AMKER, IR 83668-35-2-2-2, AYAAR, IR 68144-2B-2-2-3-1-127, NGOBANYO RED COVER, IR 91167-133-1-1-2-3, MAIGOTHI, Sarjoo-52, Barani Deep, IR-64 and GOPALBHOK (LOCAL) (Fig. 1A), while *SIZ 1* conferred innate immunity, regulation of plant growth, drought responses and freezing tolerance (Kurepa *et al.*, 2003) showed presence of bands of 205 bp and *DRF1* – an ABA

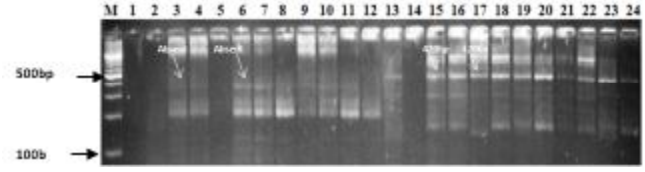

Fig. 1A : HRD.

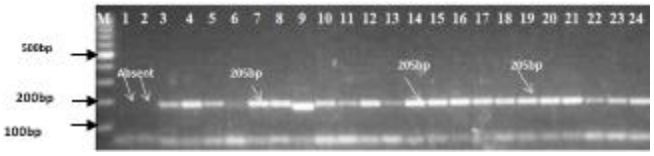

Fig. 1B : SIZ1.

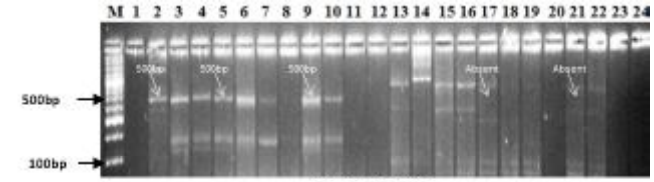

Fig. 1C : WRKY38.

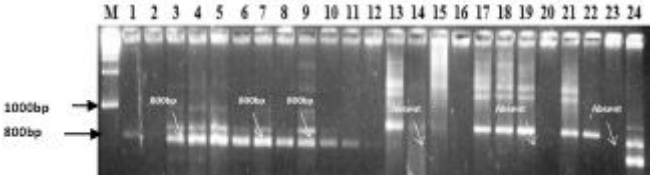

Fig. 1D : POM1.

Fig. 1E : Snac1.

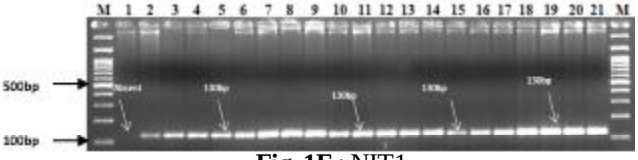

Fig. 1F : NIT1.

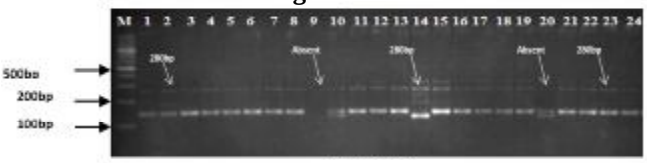

Fig. 1G : HYD3.

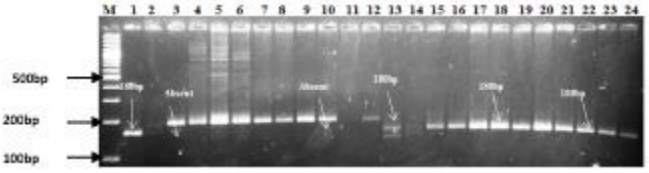

Fig. 1H : PIN3.

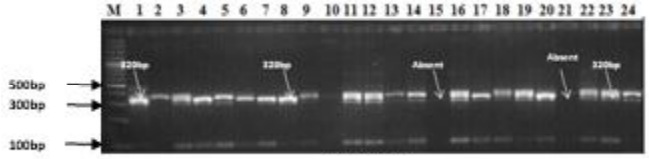

Fig. 1I : NAC2.

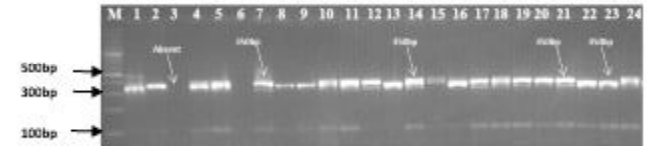

Fig. 1J : SHN1.

Fig. 1A-J : Presence/absence of ten drought responsive candidate genes in *Oryza sativa* L. species varieties. M – 1000 bp Ladder, 1.Pusa Basmati-1, 2. NDR-359, 3. NDR-97, 4. NDR-118, 5. Shusk Samrat, 6. NDR-1, 7. Nagina-22, 8. KUHUSOI-RI-SAREKU, 9.IR91167-31-3-1-33, 10.R-RHZ-2, 11.IR 92960-75-1-3, 12.IR 68144-2B-2-2-3-1-120, 13. AMKER, 14.TARAMON, 15. IR83668-35-2-2-2, 16.AYAAR, 17. IR68144-2B-2-2-3-1-127, 18. NGOBANYO RED COVER, 19. IR91167-133-1-1-2-3, 20. MAIGOTHI, 21. Sarjoo-52, 22.BaraniDeep, 23. IR-64, 24.GOPALBHOK (Local).

inducible late-embryogenesis abundant protein gene (Xue and Loverridge, 2004) presence of bands of 190 bp in all of the varieties except two viz; NDR-359 and Pusa basmati-1(Figs. 1B and 1K, respectively). *Wrky 38* involved in cold- and drought response in barley (Mare *et al*., 2004) amplify with 500 bp in eight varieties namely NDR-359, NDR-97, NDR-118, Shusk Samrat, NDR-1, Nagina-22, IR 91167-31-3-1-33 and R-RHZ-2 (Fig. 1C).

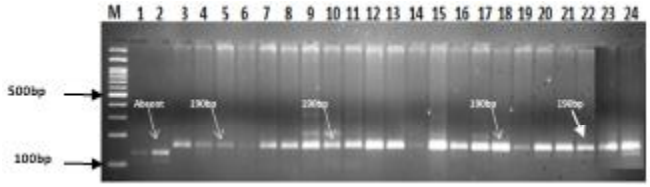

Fig. 1K : DRF1.

Fig. 1K : Presence/absence of DRF1 drought responsive candidate genes in *Oryza sativa* L. species varieties. M – 1000 bp Ladder 1.NDR-359, 2. Pusa Basmati-1, 3.NDR-118, 4.NDR-97, 5.IR 91167-31-3-1-33, 6.Shusk Samrat, 7.Nagina-22, 8.NDR-1, 9.NDR-359, 10.Sarjoo-52, 11.R-RHZ-2, 12.IR 92960-75-1-3, 13.IR 68144-2B-2-2-3-1-120, 14.Amker, 15.Taramon, 16.IR 83668-35-2-2-2, 17.AYAAR, 18.IR68144-2B-2-2-3-1-127, 19.NGOBANYO RED COVER, 20.IR91167-133-1-1-2-3, 21.MAIGOTHI, 22.IR-64, 23. GOPALBHOK (Local), 24. Barani Deep.

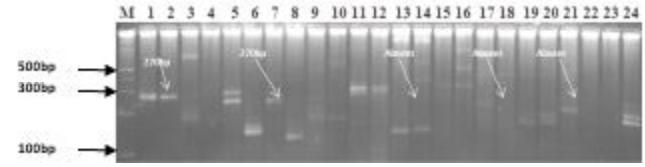

Fig. 1L : ZF1.

Fig. 1L : Presence/absence of ZF1 drought responsive candidate genes in *Oryza sativa* L. species varieties. M – 1000 bp Ladder (M) 1. Shusk Samrat, 2. NDR-1, 3. Pusa Basmati-1, 4. NDR-118, 5. Nagina-22, 6. NDR-97, 7. NDR-359, 8.KUHUSOI-RI-SAREKU, 9.IR 91167-31-3-1-33, 10.R-RHZ-2, 11.IR92960-75-1-3, 12.IR68144-2B-2-2-3-1-120, 13.AMKER, 14.TARAMON, 15.IR83668-35-2-2-2, 16.AYAAR, 17.IR 68144-2B-2-2-3-1-127, 18. NGOBANYO RED COVER, 19.IR 91167-133-1-1-2-3, 20. MAIGOTHI, 21. Sarjoo-52, 22. Barani Deep, 23. IR-64, 24. GOPALBHOK (Local).

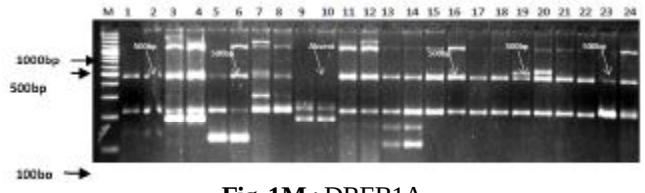

Fig. 1M : DREB1A.

Fig. 1M : Presence/absence of DREB1A drought responsive candidate genes in *Oryza sativa* L. species variety. M – 1000 bp Ladder1. NDR-97, 2. Pusa Basmati-1, 3. NDR-118, 4. KUHUSOI-RI-SAREKU, 5. IR91167-31-3-1-33, 6. Shusk Samrat, 7. Nagina-22, 8. NDR-1, 9. NDR-359, 10. Sarjoo-52, 11. R-RHZ-2, 12.IR 92960-75-1-3, 13.IR 68144-2B-2-2-3-1-120, 14.AMKER, 15. TARAMON, 16.IR 83668-35-2-2-2, 17. AYAAR, 18. IR 68144-2B-2-2-3-1-127, 19. NGOBANYO RED COVER, 20. IR91167-133-1-1-2-3, 21. MAIGOTHI, 22. IR-64, 23. GOPALBHOK (Local), 24. Barani Deep.

Table 3 : List of candidate genes and amplified polymorphic bands specific to *O. sativa* L. species.

S. no.	Name of varieties	Presence (P) or Absence (A) of genes belonging to ABA independent pathway													
		HRD	***SIZ1***	***Wrky-38***	***POM1***	***Snac-1***	***NIT1***	***HYD3***	***PIN3***	***NAC2***	***SHN1***	***DRF1***	***ZF1***	***DREB 1A***	***Hep2***
1.	**Pusa Basmati-1**	A	A	A	A	A	A	P	P	P	P	A	A	A	A
2.	**NDR-359**	A	A	P	A	A	P	P	A	A	P	A	A	P	A
3.	**NDR-97**	A	P	P	P	P	P	P	A	P	A	P	P	P	A
4.	**NDR-118**	A	P	P	P	P	P	P	A	P	P	P	A	P	A
5.	**ShuskSamrat**	A	P	P	P	P	P	P	A	P	A	P	P	P	A
6.	**NDR-1**	A	P	P	P	P	P	P	A	P	P	P	P	P	A
7.	**Nagina-22**	A	P	P	P	P	P	P	A	P	P	P	P	P	A
8.	**KUHUSOI-RI-SAREKU**	A	P	A	P	A	P	P	A	P	P	P	A	P	A
9.	**IR 91167-31-3-1-33**	A	P	P	P	P	P	A	A	A	P	P	A	P	A
10.	**R-RHZ-2**	A	P	P	P	P	P	P	A	A	P	P	A	P	A
11.	**IR 92960-75-1-3**	A	P	A	P	A	P	P	A	P	P	P	A	P	A
12.	**IR 68144-2B-2-2-3-1-120**	A	P	A	P	P	P	P	P	P	P	P	A	P	A
13.	**AMKER**	P	P	A	A	P	P	P	P	P	P	P	A	P	A
14.	**TARAMON**	A	P	A	A	P	P	P	P	P	P	P	A	P	A
15.	**IR 83668-35-2-2**	P	P	A	A	A	P	P	P	A	P	P	A	P	A
16.	**AYAAR**	P	P	A	A	P	P	P	P	P	P	P	A	P	A
17.	**IR 68144-2B-2-2-3-1-127**	P	P	A	A	P	P	P	P	P	P	P	A	P	A
18.	**NGOBANYO RED COVER**	P	P	A	A	A	P	P	P	P	P	P	A	P	A
19.	**IR 91167-133-1-1-2-3**	P	P	A	A	A	P	P	P	P	P	P	A	P	A
20.	**MAIGOTHI**	P	P	A	A	P	P	A	P	P	P	P	A	P	A
21.	**Sarjoo-52**	P	P	A	A	P	P	P	P	A	P	P	A	A	A
22.	**Barani Deep**	P	P	A	A	P	P	P	P	P	P	P	A	P	A
23.	**IR-64**	P	P	A	A	A	P	P	P	P	P	P	A	P	A
24.	**GOPALBHOK**	P	P	A	P	A	P	P	P	P	P	P	A	P	A

POM1 essential for tolerance to heat, salt and drought stresses (Rodriguez *et al*., 2012) showed 800bp amplicon in eleven varieties of rice namely NDR-97, NDR-118, ShuskSamrat, NDR-1, Nagina-22, KUHUSOI-RI-SAREKU, IR 91167-31-3-1-33, R-RHZ-2, IR 92960-75-1-3, IR 68144-2B-2-2-3-1-120 and GOPALBHOK (LOCAL) (Fig. 1D). *Snac1* gene specifically reduced water loss in rice by increasing stomatal sensitivity to ABA (Hu *et al*., 2006), showed presence of bands of 210 bp in NDR-97, NDR-118, ShuskSamrat, NDR-1, Nagina-22, IR 91167-31-3-1-33, R-RHZ-2, IR 68144-2B-2-2-3-1-120, AMKER, TARAMON, AYAAR, IR 68144-2B-2-2-3-1-127, MAIGOTHI, Sarjoo-52, Barani Deep (Fig. 1E). *NIT 1* – a crucial enzyme in auxin biosynthesis (Hillebrand *et al*., 1998), amplify with 130 bp specific band in all varieties except Pusa Basmati-1 (Fig. 1F), it is a highly drought susceptible but also develop for abiotic stress, sheath blight resistant and aroma production. *HYD3* (Beta carotene hydrolyase) conferring drought and oxidative stress resistance (Du *et al*., 2010) in barely also present in almost all rice varieties with 280bp band (Fig. 1G); but not amplified in two varieties IR 91167-31-3-1-33 and MAIGOTHI are drought susceptible variety. *PIN 3* gene that produced a 180 bp band in thirteen varieties (Fig. 1H) *viz*., Pusa Basmati-1, AMKER, TARAMON, IR83668-35-2-2-2, AYAAR, IR 68144-2B-2-2-3-1-127, NGOBANYO RED COVER, IR 91167-133-1-1-2-3, MAIGOTHI, Sarjoo-52, Barani Deep, IR-64 and GOPALBHOK (LOCAL) is a regulator of auxin efflux that was found to decrease in the presence of alloxan (Galweiler *et al*., 1998). *NAC 2* gene that showed 320 bp band in NDR-359, IR 91167-31-3-1-33, R-RHZ-2, .IR 83668-35-2-2-2 and sarjoo-52 (Fig. 1I) conferred cold and salt tolerance in rice (Hu *et al*., 2008). *SHN 1* gene was involved in wax biosynthesis (Asaph *et al*., 2004) clearly showed presence of bands of 350 bp (Fig. 1J) in all varieties except NDR-97 and NDR-1. Zinc finger protein 1 (*ZF1*) inducing abiotic stress proteins (Verma *et al*., 2013) in rice showed presence of bands of 270 bp in only four drought tolerant varieties namely, Shusk Samrat, NDR-1, Nagina-22 and NDR-97 (Fig. 1L). *DREB 1A* that exhibited 500 bp amplicon in most varieties specially in drought tolerant variety (Fig. 1M) was reported to induce a set of abiotic stress responsive genes and maintenance of water balance in plant systems (Spurthi *et al*., 2009), showed presence of bands in twenty two varieties out of all twenty four, not amplify in two varieties namely NDR-359 and sarjoo-52. In contrast, one specific genes *viz*., *Hep2* - a drought tolerant gene identified in rice (Priji and Hemaprabha, 2014) failed to amplify in this species. The absence of these genes with proven role in drought tolerance in related crops in *O. sativa* and their presence in the drought tolerant *O. sativa* species highlighted the role of these genes in imparting drought and susceptibility to drought tolerance in rice.

Conclusion

In the present investigation, eleven out 14 candidate genes belonging to ABA independent pathway of drought tolerance exhibited their presence in *O. sativa* species. The existence of a few drought responsive genes in susceptible accessions of *O. sativa* and absence of some drought specific genes in tolerant species clones were noticed. This observation leads to the existence of a complex machinery involving several genes in multiple pathways that finally makes a genotype susceptible or tolerant to drought. Also these genes have specific roles in adaptive mechanisms to survive in the long duration spanning round the year in the place of their origin/diversity.

Specifically the varieties *viz*., Nagina-22, Shusksamrat, NDR-1, NDR-97, Baranideepare the best varieties for drought tolerance for being good repositories of thirteen drought specific genes. Hence, these varieties would gain importance in pre-breeding programs aimed at developing rice varieties for drought tolerance and for isolation of novel drought specific genes. Efforts are on to identify and sequence the candidate genes that would pave way for developing an effective breeding approach for drought tolerance in rice.

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