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Identification of new alleles resistant to moisture stress in mutant lines of wheat genotypes developed by chemical mutagenesis through drought-specific ISSR markers

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Supplementary data

Table 1 — List of ISSR primers and their sequences used for the analysis of wheat germplasm

S. No.	Primer Code Name	Primer Sequence 5'-3'	Position in the genome	References
1.	UBC-810	GAGAGAGAGAGAGAGAT	1A,1B,1D,2A,2B,2D,3A.3B,3D,4A,4B,4D,5A,5B,5D,6A,6B,6D,7A,7B,7D	Hajiyev *et al*., 2021[36]
2.	UBC-812	GAGAGAGAGAGAGAGAA	1A,1B,1D,2A,2B,2D,3A.3B,3D,4A,4B,4D,5A,5B,5D,6A,6B,6D,7A,7B,7D	Hajiyev *et al*., 2021[36]
3.	UBC-814	CTCTCTCTCTCTCTCTA	1A,1B,1D,2A,2B,2D,3A.3B,3D,4A,4B,4D,5A,5B,5D,6A,6B,6D,7A,7B,7D	Son *et al*., 2013[40]
4.	UBC-815	CTCTCTCTCTCTCTCTG	1A,1B,1D,2A,2B,2D,3A.3B,3D,4A,4B,4D,5A,5B,5D,6A,6B,6D,7A,7B,7D	Khaled *et al*., 015[39]
5.	UBC-823	TCTCTCTCTCTCTCTCC	1A,1B,1D,2A,2B,2D,3A.3B,3D,4A,4B,4D,5A,5B,5D,6A,6B,6D,7A,7B,7D	Sen *et al*., 2017[5]
6.	UBC-826	ACACACACACACACACC	1A,1B,1D,2A,2B,2D,3A.3B,3D,4A,4B,4D,5A,5B,5D,6A,6B,6D,7A,7B,7D	Khaled *et al*.,2015[39]
7.	UBC-834	AGAGAGAGAGAGAGAGCT	1A,1B,1D,2A,2B,2D,3A.3B,3D,4A,4B,4D,5A,5B,5D,6A,6B,6D,7A,7B,7D	Hajiyev *et al*., 2021[36]
8.	UBC-840	GAGAGAGAGAGAGAGACT	1A,1B,1D,2A,2B,2D,3A.3B,3D,4A,4B,4D,5A,5B,5D,6A,6B,6D,7A,7B,7D	Son *et al*., 2013[40]
9.	UBC-845	CTCTCTCTCTCTCTCTAG	1A,1B,1D,2A,2B,2D,3A.3B,3D,4A,4B,4D,5A,5B,5D,6A,6B,6D,7A,7B,7D	Sen *et al*., 2017[5]
10.	UBC-848	CACACACACACACACAAG	1A,1B,1D,2A,2B,2D,3A.3B,3D,4A,4B,4D,5A,5B,5D,6A,6B,6D,7A,7B,7D	Son *et al*., 2013[40]
11.	UBC-852	TCTCTCTCTCTCTCTCGA	1A,1B,1D,2A,2B,2D,3A.3B,3D,4A,4B,4D,5A,5B,5D,6A,6B,6D,7A,7B,7D	Sen *et al*., 2017[5]
12.	UBC-853	TCTCTCTCTCTCTCTCAT	1A,1B,1D,2A,2B,2D,3A.3B,3D,4A,4B,4D,5A,5B,5D,6A,6B,6D,7A,7B,7D	Sen *et al*., 2017[5]
13.	UBC-855	ACACACACACACACACCT	1A,1B,1D,2A,2B,2D,3A.3B,3D,4A,4B,4D,5A,5B,5D,6A,6B,6D,7A,7B,7D	Khaled *et al*.,2015[39]
14.	UBC-876	GATAGATAGAGAGACA	1A,1B,1D,2A,2B,2D,3A.3B,3D,4A,4B,4D,5A,5B,5D,6A,6B,6D,7A,7B,7D	Khaled *et al*.,2015[39]
15.	UBC-881	GGGTGGGGTGGGGGTG	1A,1B,1D,2A,2B,2D,3A.3B,3D,4A,4B,4D,5A,5B,5D,6A,6B,6D,7A,7B,7D	Khaled *et al*.,2015[39]

Note: Selected ISSR markers were screened to determine their chromosomal locations using BLASTn on NCBI. The results revealed that all ISSR primers were successfully mapped to chromosomes, as described in the table. BLAST analysis showed 100% similarity, confirming that all primers are located on the chromosomes of wheat cultivars. These selected primers were then used for genetic diversity analysis and further molecular analysis in mutants.

Table 2 — List of DNA amplification components for Primers

S. No.	Components	Volume (µL)
1.	10X Assay Buffer with 15.0 mM $MgCl_2$	1.6
2.	1.0 mM dNTPs Mix	1.0
3.	Taq DNA Polymerase (1U/µL)	0.5
4.	5.0 µM Primer	1.0
5.	DNA (25ng/µL)	1.2
6.	Water (Milli Pore)	9.7
	Total	15 µL

Table 3 — DNA amplification reaction condition for PCR

S. No.	Steps	Temperature (°C)	Time
1.	Initial denaturation	95.0	5 min
2.	Denaturation	94.0	1 min
3.	Annealing	Variable with primer	30 sec
4.	Extension	72.0	1 min
5.	Cycles	35.0	……
6.	Final extension	72.0	7.0 min
7.	Final hold	04.0	1 h

Table 4 — Primer code, number of amplified bands, mono and polymorphic alleles, PIC value and resolving power of 18 wheat normal and mutant plant of HD 3226 variety

Primers	Total Number of alleles	Number of amplified bands	Polymo - rphic alleles	Monom -orphic alleles	Polymorphism %	PIC value		Marker index	Resolving power
						EMS	SA		
UBC 810	5	74	3	2	60.00	0.22	0.27	0.73	8.22
UBC 814	3	25	3	0	100.00	0.72	0.34	1.59	2.78
UBC 815	4	54	4	0	100.00	0.38	0.36	1.48	6.00
UBC 823	4	57	2	2	50.00	0.25	0.34	0.59	6.33
UBC 826	2	36	0	2	0.00	0.00	0.00	0.00	4.00
UBC 834	3	21	3	0	100.00	0.73	0.39	1.68	2.33
UBC 840	6	86	4	2	66.67	0.31	0.31	1.24	9.56
UBC 845	6	51	6	0	100.00	0.80	0.52	3.96	5.67
UBC 848	3	31	3	0	100.00	0.66	0.79	2.17	3.44
UBC 852	2	23	2	0	100.00	0.59	0.47	1.06	2.55
UBC 853	4	56	4	0	100.00	0.46	0.21	1.34	6.22
UBC 855	3	45	3	0	100.00	0.26	0.37	0.95	5.00
UBC 881	5	26	5	0	100.00	0.69	0.81	3.75	2.89
Total	50	585	42	8					
					Average	0.47	0.39	1.58	4.99

Table 5 — Primer code, number of amplified bands, mono and polymorphic alleles, PIC value and resolving power of 10 wheat normal and mutant plant of HI-1620 variety

Primers	Total Number of alleles	Number of amplified bands	Polymo - rphic alleles	Monom - orphic alleles	Polymorphism %	PIC value		Marker index	Resolving power
						EMS	SA		
UBC 810	5	35	3	2	60.00	0.22	0.32	0.81	7.00
UBC 814	3	17	2	1	66.67	0.18	0.51	0.69	3.40
UBC 815	4	26	4	0	100.00	0.36	0.29	1.30	5.20
UBC 823	2	18	2	0	100.00	0.00	0.30	0.30	3.60
UBC 826	2	20	0	2	0.00	0.00	0.00	0.00	4.00
UBC 834	2	13	2	0	100.00	0.52	0.59	1.11	2.60
UBC 840	4	33	4	0	100.00	0.41	0.18	1.18	6.60
UBC 845	5	28	5	0	100.00	0.32	0.78	2.75	5.60
UBC 848	3	7	3	0	100.00	0.89	0.64	2.29	1.40
UBC 852	2	12	2	0	100.00	0.55	0.55	1.10	2.40
UBC 853	4	31	2	2	50.00	0.18	0.37	0.55	6.20
UBC 855	3	22	2	1	66.67	0.29	0.42	0.71	4.40
UBC 881	2	11	2	0	100.00	0.65	0.52	1.17	2.20
Total	41	273	33	8					
					Average	0.35	0.42	1.07	4.2

Table 6 — Jaccard's similarity coefficients based on ISSR marker analysis in HD-3226 wheat genotype's mutants

	C1	C2	Mt1	Mt2	Mt3	Mt4	Mt5	Mt6	Mt7	Mt8	Mt9	Mt10	Mt11	Mt12	Mt13	Mt14	Mt15	Mt16
C1	1																	
C2	1	1																
Mt1	0.69	0.69	1															
Mt2	0.666	0.667	0.595	1														
Mt3	0.761	0.761	0.69	0.667	1													
Mt4	0.69	0.69	0.714	0.738	0.785	1												
Mt5	0.619	0.619	0.5	0.619	0.761	0.69	1											
Mt6	0.547	0.547	0.667	0.452	0.452	0.571	0.404	1										
Mt7	0.428	0.428	0.595	0.476	0.476	0.547	0.476	0.547	1									
Mt8	0.595	0.595	0.619	0.69	0.547	0.619	0.595	0.476	0.69	1								
Mt9	0.547	0.547	0.761	0.5	0.642	0.714	0.547	0.619	0.785	0.619	1							
Mt10	0.547	0.547	0.714	0.595	0.547	0.619	0.452	0.619	0.5	0.667	0.667	1						
Mt11	0.667	0.667	0.595	0.667	0.809	0.738	0.667	0.5	0.571	0.547	0.738	0.547	1					
Mt12	0.642	0.642	0.619	0.642	0.69	0.571	0.5	0.523	0.5	0.619	0.667	0.667	0.69	1				
Mt13	0.523	0.523	0.642	0.571	0.571	0.642	0.523	0.5	0.761	0.69	0.738	0.5	0.667	0.547	1			
Mt14	0.667	0.667	0.642	0.571	0.857	0.69	0.667	0.5	0.523	0.5	0.642	0.5	0.714	0.595	0.571	1		
Mt15	0.714	0.714	0.833	0.571	0.761	0.833	0.667	0.624	0.619	0.642	0.833	0.642	0.714	0.595	0.667	0.714	1	
Mt16	0.69	0.69	0.571	0.69	0.88	0.714	0.69	0.523	0.452	0.571	0.571	0.571	0.738	0.761	0.5	0.785	0.69	1

Genotype HD- 3226:-C1-Control with water, C2- Control with 15% PEG, Mt1-0.25% EMS (water, P-21), Mt2-0.25% EMS (water, P-10), Mt3-0.25% EMS (15% PEG, P-19), Mt4-0.5% EMS (water, P-49), Mt5-0.5% EMS (15% PEG, P-41), Mt6-0.75% EMS (15% PEG, P-63), Mt7-0.75% EMS (15% PEG, P-67), Mt8-1.0% EMS (15% PEG, P-19), Mt9-1.0% EMS (15% PEG, P-25), Mt10-0.02% SA (water, P-4), Mt11-0.02% SA (15% PEG, P-28), Mt12-0.02% SA (15% PEG, P-55), Mt13-0.04% SA (water, P-7), Mt14-0.04% SA (15% PEG, P-2), Mt15-0.04% SA (15% PEG, P-43) and Mt16-0.04% SA (15% PEG, P-36).

Table 7 — Jaccard's similarity coefficients based on ISSR marker analysis in HI-1620 wheat genotype's mutants

	C1	C2	Mt1	Mt2	Mt3	Mt4	Mt5	Mt6	Mt7	Mt8
C1	1									
C2	0.97	1								
Mt1	0.676	0.676	1							
Mt2	0.764	0.764	0.705	1						
Mt3	0.617	0.617	0.558	0.59	1					
Mt4	0.5	0.5	0.617	0.53	0.5	1				
Mt5	0.5	0.5	0.558	0.41	0.44	0.441	1			
Mt6	0.735	0.735	0.617	0.82	0.56	0.5	0.444	1		
Mt7	0.47	0.47	0.588	0.44	0.47	0.529	0.764	0.57	1	
Mt8	0.529	0.529	0.529	0.62	0.71	0.529	0.588	0.588	0.617	1

Genotype HI-1620:-C1- Control with water, C2- Control with 15% PEG, Mt1-0.25% EMS (water, P-11), Mt2- 0.25% EMS (15% PEG, P-4), Mt3- 0.25% EMS (15% PEG, P-45), Mt4- 1.0% EMS (15% PEG, P-21), Mt5- 0.02% SA (15% PEG, P-28), Mt6- 0.02% SA (15% PEG, P-4), Mt7- 0.08% SA (15% PEG, P-1) and Mt8- 0.08% SA (15% PEG, P-2).

Table 8 — Selected M_3 twenty four mutant plants shows variations in their banding patterns of genomic DNA using ISSR markers

S. No.	Mutants	Variation at marker sites
1	EMS-0.25% HD-3226 WATER (P-21)	UBC 810,UBC 815,UBC 840,UBC 845,UBC 848,UBC 852,UBC 853,UBC 855 and UBC 881
2	EMS-0.25% HD-3226 WATER (P-10)	UBC 834,UBC 840 and UBC 881
3	EMS-0.25% HD-3226 15% PEG (P-19)	UBC 840,UBC 845,UBC 848 and UBC 852
4	EMS-0.5% HD-3226 WATER (P-49)	UBC 814,UBC 834,UBC 845,UBC 848,UBC 852 and UBC 881
5	EMS-0.5% HD-3226 15% PEG (P-41)	UBC 810,UBC 823,UBC 845,UBC 848,UBC 852,UBC 853 and UBC 881
6	EMS-0.75% HD-3226 15% PEG (P-63)	UBC 810, UBC 814, UBC 815,UBC 840,UBC 848,UBC 852 and UBC 881
7	EMS-0.75% HD-3226 15% PEG (P-67)	UBC 823,UBC 840,UBC 845,UBC 848,UBC 852,UBC 853 and UBC 881
8	EMS-1.0% HD-3226 15% PEG (P-19)	UBC 840,UBC 845,UBC 853 and UBC 881
9	EMS-1.0% HD-3226 15% PEG (P-25)	UBC 840,UBC 845,UBC 848,UBC 852,UBC 853and UBC 881
10	SA- 0.02% HD-3226 WATER (P-4)	UBC 823,UBC 840,UBC 845,UBC 853,UBC 855 and UBC 881
11	SA- 0.02% HD-3226 15% PEG (P-28)	UBC 823,UBC 845,UBC 848,UBC 852 and UBC 853
12	SA- 0.02% HD-3226 15% PEG (P-55)	UBC 815,UBC 823,UBC 845,UBC 853,UBC 855 and UBC 881
13	SA- 0.04% HD- 3226 WATER (P-7)	UBC 810,UBC 845,UBC 848,UBC 852,UBC 853,UBC 855 and UBC 881
14	SA- 0.04% HD- 3226 15% PEG (P-2)	UBC 810,UBC 823,UBC 845,UBC 848,UBC 852 and UBC 853
15	SA- 0.04% HD-3226 15% PEG (P-43)	UBC 810,UBC 823, UBC 840,UBC 845,UBC 848,UBC 852,UBC 853and UBC 881
16	SA- 0.04% HD-3226 15% PEG (P-36)	UBC 814, UBC 815, UBC 840, UBC 845, UBC 848, UBC 852, UBC 853 and UBC 855
17	EMS-0.25% HI- 1620 WATER (P-11)	UBC 810,UBC 815, UBC 848,UBC 852,UBC 855 and UBC 881
18	EMS-0.25% HI- 1620 15% PEG (P-4)	UBC 810,UBC 845,UBC 853 and UBC 855
19	EMS-0.25% HI- 1620 15% PEG (P-45)	UBC 810, UBC 815, UBC 823, UBC 845, UBC 848, UBC 852, UBC 855 and UBC 881
20	EMS-1.0% HI- 1620 15% PEG (P-21)	UBC 840,UBC 845,UBC 848,UBC 852,UBC 855 and UBC 881
21	SA- 0.02% HI- 1620 15% PEG (P-28)	UBC 810,UBC 815, UBC 840,UBC 845,UBC 853,UBC 855 and UBC 881
22	SA- 0.02% HI- 1620 15% PEG (P-4)	UBC 810,UBC 823,UBC 845 and UBC 853
23	SA- 0.08% HI- 1620 15% PEG(P-1)	UBC 810,UBC 814, UBC 815,UBC 823, UBC 840,UBC 845,UBC 853 and UBC 881
24	SA- 0.08% HI- 1620 15% PEG (P-2)	UBC 810, UBC 823, UBC 840,UBC 845,UBC 848,UBC 852 and UBC 881