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Genome-wide Association Mapping (GWAS) for Anther Extrusion in Bread Wheat (*Triticum aestivum* L.) Using DArT-SNP Markers

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Abstract

Association Mapping (AM) for anther extrusion trait was done in a sub-set of 190 diverse genotypes of SWRS material grown at University Research Farm and showed a wide range of variation. The growth habit, country of origin and AE (Anther extrusion) scores of all 190 genotype. The range of AE score of the 190 wheat genotypes differ from 0.47 to 2.97 with a mean value of 1.23 and a SD of 0.36 %. In association analysis based on mean values over environments, significant association ($p<0.01$) of different traits (AE, DTH, DTA, DTM). DTH was observed for a total of 181 DArT SNP marker loci following GLM. Anther Extrusion was observed for a total of 177 DArT SNP marker loci following GLM.

Key Words: *Triticum aestivum* L, MTA, Agronomic traits, DArT-SNP

Introduction

Bread wheat (*Triticum aestivum* L) is one of the most important domesticated food crops of the world. It occupies 17% (one sixth) of crop acreage world over, and about ~771 million tons total production in the world and ~95 million tons in the India (FAOSTAT Database). Association mapping analysis, an alternative method, has been used extensively in a variety of organism to identify marker-trait associations (MTAs) (DeWan *et al*., 2006; Karlsson *et al.*, 2007) and uses natural populations or germplasm collections instead of segregating bi-parental populations. In wheat, results of several AM studies have been reported (Jing *et al.*, 2007; Peng *et al.*, 2009, Kulwal *et al.*, 2012; Mir ***et al.***, 2012).

In bread wheat, numerous studies have been performed to investigate pollen dispersal outside the floret. It appears to depend on anther extrusion, size of anthers, the opening of the glumes, the awnless of the lemma, the size of the stigma, the duration of the stigma receptivity, the number of pollen grains per anther, the longevity of the pollen grain, and other factors (Langer *et al.*, 2014; Boeven *et al.*, 2016; Muqaddasi *et al.*, 2017).

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Wheat belongs to subtribe Triticinae, tribe Triticeae of family Poaceae. It is a segmental allohexaploid (2n = 6x = 42), with comprises diploid (2n=2×=14; AA), tetraploid (2n=4×=28; AABB) and hexaploid (2n=6×=42; AABBDD) species. The hexaploid wheat, which is also known as 'bread wheat' or as 'common wheat', originated through natural hybridization of three diploid species in two stages, 1) *T. urartu* (AA genome) hybridized with an unknown diploid species probably *Aegilops speltoides* (BB genome) that generate tetraploid wheat (AABB genome) after chromosomes doubling followed by 2) hybridization with a third ancestral species, *A. tauschii* (DD genome). It is well known that the three sub-genomes of hexaploid wheat are organized into seven homoeologous (partial homologous) groups, each homoeologous group having three closely related homoeologous chromosomes, one each from the three sub-genomes (Kihara, 1944). Bread wheat has genome size of 16 Gb which ~8-fold larger than that of maize and 40-fold larger than that of rice (Bennett and Leitch 2005).

Materials and Methods

Genetic materials

The association mapping panel comprised sub-set of 190 diverse genotypes of a spring wheat reference set (SWRS) of bread wheat procured from CIMMYT gene bank, Mexico (Appendix 1).

Genotyping data and markers

A set of 17,937 polymorphic SNP markers generated using DArT-seq, at Diversity Array Technology Pvt. Ltd. Australia under the "Seed for Discovery" project of CIMMYT Mexico, was used for genotyping of all the 246 accessions of bread wheat. The markers were mapped on chromosomes using DArT PL's consensus map of wheat based on >100 crosses. The map (version 4) has 110,000 markers including ~5,000 original DArT markers, the remaining being DArTseq markers. These markers are spreaded over all the 21 chromosomes. The 8637 SNPs were mapped on chromosomes, of which 2973 SNPs belonged to a sub-genome, 4505 SNPs belonged to the B sub-genome and 1159 SNPs belonged to the D sub-genome and remaining 9300 SNPs were unmapped.

Field experiment

The SWRS (Spring Wheat Reference Set) populations having 190 selected genotypes were sown on 12 November, 2018. Seeds of the Wheat lines were hand dibbled in dry soil, each genotype was raised in a plot comprising three lines, each line being 1.5 m long with line-to-line distance of 20 cm, genotype to genotype distance was carried 40 cm of each genotype in two replications under simple lattice design. The uniform plant stand was maintained by thinning and gap filling. NPK fertilizers were applied at the rate of 100:50:50 kg/ha. While P and K were applied basally during sowing, N was applied in three splits as top dressing, first ½ parts during sowing and 1/4-1/4 part two times after CRI stage and during heading. Insect and weed control measures were applied periodically as required.

Recording of data on anther extrusion

The plants of 190 wheat genotypes were evaluated in anther retention (AR; number of non-extruded anthers) score on field in 5–10 days post-anthesis in February and March 2019 by observing the anthers retained inside the four pairs of primary and secondary florets sampled from the central portion of four spikes per plot. Anthers extrusion was calculated by subtracting the number of retained (non-extruded) anthers from 24, since the total number of anthers housed by eight florets is 24 (4 spikelets × 2 lateral florets × 3 anthers) as described in Muqaddasi *et al.*, (2016).

Statistical analysis of the phenotypic data

The estimates of descriptive statistics including mean, range, standard error, correlation and distribution of genotypes for anther extrusion trait were performed using SPSS version 17.0

Population structure analysis

The model-based methods have been more dominant as procedures for inference about population structure, mostly with implementation of Bayesian clustering and maximum-likelihood techniques in programs such as STRUCTURE, ADMIXTURE (Alexander *et al*., 2009).

GWAS analysis using GLM (General Linear Model) and MLM (Mixed Linear Model)

TASSEL v. 3.0 was used for this purpose using both General Linear Model (GLM) and Mixed Linear Model (MLM). For GLM, population structure (the Q model) without familial

relatedness (the K model) was used, whereas for MLM, both population structure and the familial relatedness (Q + K model) were used (**Yu *et al.*, 2006**). The general equations for GLM and MLM were y = Xa + e and y = Xa + Qb + Zu + e, respectively (**Tadesse *et al.*, 2015**), where y is the vector for phenotypes, a is the vector for marker fixed effects, b is the vector of fixed effects, u is the vector of random effects (the kinship matrix) and e is the vector of residuals. Distance matrix derived from cladogram function was converted into a similarity matrix using TASSEL (**Kang *et al.*, 2008**). X is the matrix for marker genotypes, Q is the Q-matrix obtained from the STRUCTURE software and Z is an identity matrix. Significance of marker-trait associations (MTAs) was determined at $P \leq 0.001$

Appendix I. A list of 190 diverse genotypes of spring wheat reference set (SWRS) that was used as the association mapping panel for GWAS.

Genotype	Name	Genotype	Name
SWRS1	ZAMBESI	SWRA43	VEE/PJN/ /2*TUI/3/WH576
SWRS2	GRAY JD738	SWRA44	MUNIA/3/RUFF/FGO/YAV79/4/PASTOR
SWRS3	GRAY JD930	SWRA45	(TAUS)/4/WEAVER/5/IRENA
SWRS4	GRAY JD1024	SWRA46	PBW343/TONI
SWRS5	GRAY JD1032	SWRA47	VEE#8//JUP/BJY/3/F3.71/TRM/4/2*WEAVER/5/HAHN/2*WE
SWRS6	GRAY JD1102		
SWRS7	GRAY JD1196	SWRA48	URES/BOW/ /OPATA/3/PASTOR
SWRS8	GRAY JD1278	SWRA49	CHAPIO
SWRS9	C-40-1	SWRA50	TUI
SWRS10	MOSKOVSKA	SWRA51	DUCULA
SWRS11	YA 21 VIR 48760	SWRA52	TNMU/3/JUP/BJY/ /SARA
SWRS12	W-33-A	SWRA53	BT-SCHOMBURGK
SWRS13	GONG JIAO 279	SWRA54	EXCALIBUR
SWRS14	BW110	SWRA55	BARUNGA
SWRS15	TOKSUN SPRING 2 ST-122	SWRA56	KRICHAUFF
SWRS16	YANTAGBAY	SWRA57	WESTONIA
SWRS17	CItr 4309	SWRA58	BATAVIA
SWRS18	JENKIN	SWRA59	WW425
SWRS19	MARGARITOVO	SWRA60	SITTA
SWRS20	REWARD	SWRA61	CETTLA
SWRS21	DORSETT-PH-3892	SWRA62	TUI
SWRS22	ROJO BARBON	SWRA63	JUN/BOMB
SWRS23	DORSETT-PH-6955	SWRA64	TAM200/TUI
SWRS24	DORSETT-PH-7017	SWRA65	SUPER SERI #1
SWRS25	DORSETT-PH-6927	SWRA66	CROC_1/AE.SQUARROSA(205)//KAUZ
SWRS26	DORSETT-PH-7150	SWRA67	HXL7573/2*BAU
SWRS27	Citr 14998	SWRA68	DHARWAR DRY
SWRS28	/BUC/PVN/3/YR/4/TRAP#1	SWRA69	PAVON TALL
SWRS29	KAUZ/ /ALTAR 84/AOS/3/KAUZ	SWRA70	KAUZ TALL
SWRS30	OTUS	SWRA71	NESSER TALL
SWRS31	SKAUZ82/FCT	SWRA72	PASTOR/BAV92
SWRS32	SIRKKU	SWRA73	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS
SWRS33	OTUS/TOBA97	SWRA74	BERKUT

SWRS34	BONASA	SWRS75	VOROBEY
SWRS35	KAUZ/ /BOW/NKT	SWRS76	ALTAR 84/AE.SQUARROSA (224)//2*YACO/3/BAV92
SWRS36	ECIJA:AE		
SWRS37	SW89.5181/KAUZ	SWRS77	MILAN/KAUR/ /DHARWAR DRY/3/BAV92
SWRS38	MINO	SWRS78	KABY/BAV92/3/CROC_1/AE.SQUARROSA (224)/ /OPATA
SWRS39	CHIBIA/PASTOR/ /CHIBIA		
SWRS40	VERDIN	SWRS79	BJY/COC//PRL/BOW/3/MILAN/KAUZ/4/BAV92
SWRS41	CHIBIA/PASTOR/ /CHIBIA		
SWRS80	ATTILA/BAV92/ /PASTOR	SWRS116	SAKHA 69
SWRS81	OMSKAYA-32	SWRS117	SALAMANCA 75
SWRS82	KE FENG 2	SWRS118	SAN CAYETANO S 97
SWRS83	NEW LONG MAI 19	SWRS119	TANORI F 71
SWRS84	LONG MAI 23	SWRS120	TINAMOU I1
SWRS85	AC VISTA	SWRS121	TOBARITO M 97
SWRS86	CHUM18/SERI	SWRS122	ZAMINDAR 80
SWRS87	SABUF/4/ALTAR 84/AE.SQUARROSA /YACO/3/CROC_1AE.SQUARROSA SABUF/4/ALTAR 84/AE.SQUARROSA	SWRS123	CNO/7C
		SWRS124	FIRETAIL
		SWRS125	PFAU/VEE#9/ /URES
SWRS88	PASTOR/3/KAUZ*2/OPATA/ /KAUZ	SWRS126	BJY/COC/ /PRL/BOW/3/MILAN/KAUZ/4/BAV92
SWRS89	CROC_1/AE.SQUARROSA (205)/ /KAUZ/3/SASIA		
		SWRS127	CHIL/BOMB
SWRS90	CROC_1/AE.SQUARROSA (205)/	SWRS128	REDWING
SWRS91	CROP_1/AE.SQUARROSA (205)/ /BORL95/3/ATTILA SQUARROSA (TAUS) /4/WEAVER	SWRS110	LERMA ROJO 64A
		SWRS111	OROFEN 60
		SWRS112	PAVON F 76
SWRS92	CHEN/AEGILOPS SQUARROSA (TAUS)/ /BCN/3/CMH81.38/2*KAUZ	SWRS113	PIRSABAK 85
		SWRS114	POTAM S 70
SWRS93	CROC_1/AE.SQUARROSA (205) //KAUZ/3/PASTOR	SWRS115	SAFED LERMA
		SWRS116	SAKHA 69
SWRS94	ESDA/ /ALTAR 84/AE.SQUARROSA (211)/3/ESDA/4/CHOIX	SWRS117	SALAMANCA 75
		SWRS118	SAN CAYETANO S 97
SWRS95	CROC_1/AE.SQUARROSA (205)/ /KAUZ/3/ATTILA	SWRS119	TANORI F 71
		SWRS120	TINAMOU I1
SWRS96	FISCAL	SWRS121	TOBARITO M 97
SWRS97	CHIBIA/4/PGO/ CROC_1/ AE.SQUARROSA(224)/3/2*BORL95	SWRS122	ZAMINDAR 80
		SWRS123	CNO/7C

SWRS98	EXCALIBUR	SWRS124	FIRETAIL
SWRS99	JANZ	SWRS125	PFAU/VEE#9/ /URES
SWRS100	KULIN	SWRS126	BJY/COC/ /PRL/BOW/3/MILAN/KAUZ/4/BAV 92
SWRS101	CUNNINGHAM		
SWRS102	WEAVER	SWRS127	CHIL/BOMB
SWRS103	MILAN	SWRS128	REDWING
SWRS104	CHIBIA	SWRS129	D67.2/P66.270/ /AE.SQUARROSA (320)/3/CUNNINGHAM
SWRS105	PAPAGE M 86		
SWRS106	KAUZ	SWRS130	D67.2/P66.270/ /AE.SQUARROSA (320)/3/CUNNINGHAM
SWRS107	BAW898		
SWRS108	CUMHURIYET 75	SWRS131	PARUS/3/CHEN/AE.SQ/ /2*OPATA
SWRS109	GONEN	SWRS132	ATTILA/3*BCN/3/CROC_1/AE.AQ UARROSA(224)/ /OPATA
SWRS110	LERMA ROJO 64A		
SWRS111	OROFEN 60	SWRS132	ATTILA/3*BCN/3/CROC_1/AE.AQ UARROSA
SWRS112	PAVON F 76	SWRS133	YANAC/3PRL/SARA//TSI/VEE#5/4 /CROC_1/AE.SQUARROSA (224)//OPATA
SWRS113	PIRSABAK 85		
SWRS114	POTAM S 70	SWRS134	FGO/USA2111/ /AE.SQUARROSA (658)/3/PRL/SARA//TSI/VEE#5/4/AT TILA
SWRS115	SAFED LERMA		
SWRS135	CROC_1/AE.SQUARROSA (205)/ /KAUZ/3/DHARWAR DRY/4/WBLL1	SWRS166	OAX93.10.1
		SWRS167	OAX93.10.1
SWRS136	AUS 4930.7/2*PASTOR	SWRS168	CANUCK
SWRS137	CETA/AE.SQUARROSA (327)//2*SUNLIN	SWRS169	AEGES
SWRS138	T.DICOCCON PI225332/AE.SQUARROS (895//WBILL1/3/2*WVLL1	SWRS170	ELVAS 61-37
		SWRS171	G67786
SWRS139	T.DICOCCONPI225332/AE.SQU ARROSA (895)//WBLL1/3/2*WBLL1	SWRS172	GLENLEA
		SWRS173	HYBRIDE 56 VILMORIN
SWRS140	T.DICOCCONPI225332/AE.SQU ARROSA (895)//WBLL1/3/2*WBLL1	SWRS153	BETTY/3/CHEN/AE.SQ//2*OPATA
		SWRS154	BETTY/3/CHEN/AE.SQ//2*OPATA
SWRS141	T.DICOCCON PI94625/SQUARROSA (372)/ /3*PASTOR	SWRS155	KINCI/3/CHEN/AE.SQ//2*OPATA
		SWRS156	NL90.15.2.3
SWRS142	T.DICOCCON PI94625/AE.SQUARROSA (372)/ /3*PASTOR	SWRS157	NL90.15.2.57
		SWRS158	PBL94.14.39
SWRS143	T.DICOCCONPI225332/AE.SQU ARROSA (895)//WBLL1/3/2*WBLL1	SWRS159	QRO94.2.117
		SWRS160	HGO94.9.1.3
SWRS144	CHEN/AE.SQ/ /2*OPATA/3/TRCH	SWRS161	HGO94.9.1.23

SWRS145	SCA/AE.SQUARROSA(518)/3/URES/JU/ /KAUZ/4/USES/JUN/KAUZ	SWRS162	HGO94.9.1.37
		SWRS163	HGO94.9.2.29
SWRS146	CROC_1/AE.SQUARROSA(224)/ 2*OPTTA/3/THB/CEP7780/SHA 4/LIRA/4/FRET2	SWRS164	MEX94.2.39
		SWRS165	MEX94.22.97
SWRS147	D67.2/P66.270/ /AE.SQUARROSA (220)/3/PRL/SARA/TSI/VEE#5/4/ METSO	SWRS166	OAX93.10.1
		SWRS167	OAX93.10.1
SWRS148	CETA/AE.SQUARROSA (1027)/3/URES/JUN//KAUZ/4/U RES/JUN//KAUZ	SWRS168	CANUCK
		SWRS169	AEGES
SWRS149	NING CHUN20/3/MYNA/VUL/JUN/6/F ILIN/IRENA/5C	SWRS170	ELVAS 61-37
		SWRS171	G67786
SWRS150	NING CHUN20/3/MYNA/VUL/JUN/6/F ILIN/IRENA/5C	SWRS172	GLENLEA
		SWRS173	HYBRIDE 56 VILMORIN
SWRS151	NORM.2-ABC178/3/CHEN/AE.SQ//2*OPA TA	SWRS174	KIMMO
		SWRS175	KIURU
SWRS152	NORM.2-A BC178/3/CHEN/AE.SQ//2*OPAT A	SWRS176	M-708/G25//NURSI 163
		SWRS177	MANITAL
SWRS153	BETTY/3/CHEN/AE.SQ//2*OPA TA	SWRS178	PANE-2
SWRS154	BETTY/3/CHEN/AE.SQ//2*OPA TA	SWRS179	PITIC S62
SWRS155	KINCI/3/CHEN/AE.SQ//2*OPAT A	SWRS180	SEVILLANO
SWRS156	NL90.15.2.3	SWRS181	ANZA
SWRS157	NL90.15.2.57	SWRS182	GABO
SWRS158	PBL94.14.39	SWRS183	MARCOS JUAREZ INTA
SWRS159	QRO94.2.117	SWRS184	NACOZARI F 76
SWRS160	HGO94.9.1.3	SWRS185	NEELKANT
SWRS161	HGO94.9.1.23	SWRS186	PASTOR
SWRS162	HGO94.9.1.37	SWRS187	PBW343
SWRS163	HGO94.9.2.29	SWRS188	PENJAMO 62
SWRS164	MEX94.2.39	SWRS189	SIETE CERROS T66
SWRS165	MEX94.22.97	SWRS190	TRIPLE DIRK

Results and Discussion

Phenotypic analysis

Phenotyping for anther extrusion trait was done in a sub-set of 190 diverse genotypes of SWRS material grown at University Research Farm and showed a wide range of variation. The growth habit, country of origin and AE scores of all 190 genotype are given in Table. The two methods are measure the AE in both spring (SP) and winter wheat (WP) panels were adopted, i.e., (1) direct scoring of the anthers on the field and (2) collecting and freezing the spikes and determining the AE in the laboratory. Both methods strongly correlated with one another. The laboratory-based method was preferred for the subsequent genetic

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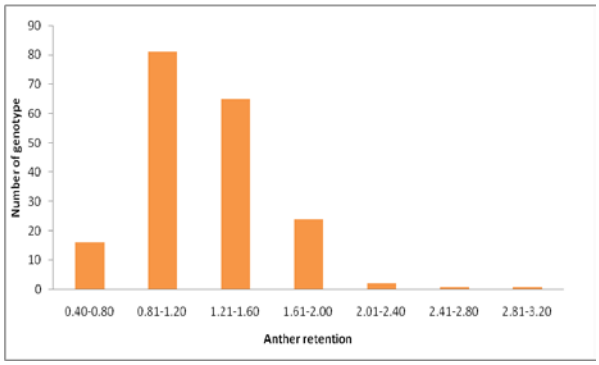
analysis, because it avoided difficulties related with the on field AE scoring and lodging of the non-dwarf accessions and because it allowed the processing of large numbers of accessions.

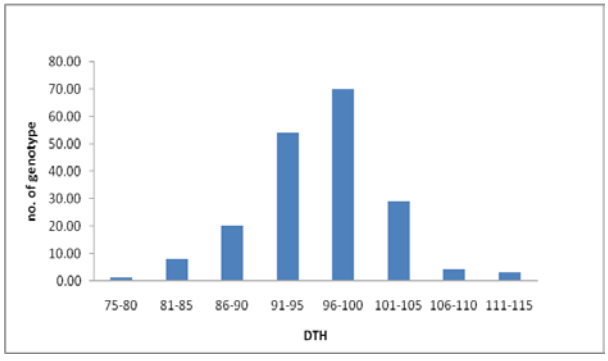
Frequency distribution of AE trait

The range of AE score of the 190 wheat genotypes differ from 0.47 to 2.97 with a mean value of 1.23 and a SD of 0.36 %. The distribution of AE score for the 190 wheat genotypes was highly skewed towards higher AE, with a large number (162) of genotypes falling in higher AE group (AE score 0.40-1.60) and 24 genotype under moderate AE (score 1.61-2.00) and 4 low AE (score 2.01-3.20) genotypes.



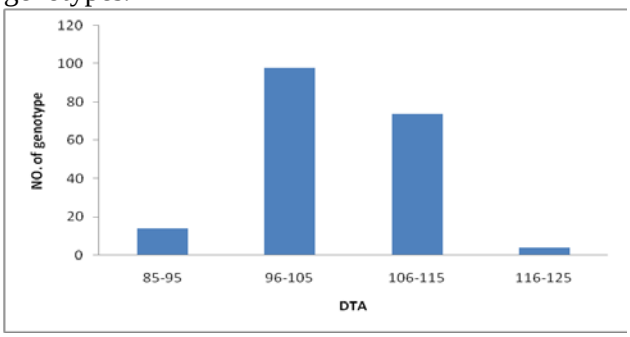
Frequency distribution of DTH trait,

The distribution of DTH score for the 190 wheat genotypes was highly skewed towards moderate DTH, with a large number (124) of genotypes falling in moderate DTH group (DTH score 91-100) and 36 genotype falling in highest DTH group (score 101-115) and 29 lowest DTH group (score 75-90) genotypes.



Frequency distribution of DTA trait

The distribution of DTA score for the 190 wheat genotypes was highly skewed towards moderate DTA group, with a large number (172) of genotypes falling in moderate DTA group (DTA score 96-115) and 4 genotype under higher DTA (116-125) and 14 under lowest DTA (score 85-95) genotypes.



Descriptive statistics for phenotypic traits

Descriptive statistics including values of mean, range, standard deviation and coefficient of variation (CV %) for AE traits are presented in Table.

Descriptive Statistics

Trait	N	Minimum	Maximum	Mean	Std. Deviation
AR	190	0.47	2.97	1.2345	0.36226

Correlations

Trait	PH	DTH	DTA	DTM	AR
PH	1	.144*	0.124	0.132	-0.065
DTH		1	.949**	.331**	-0.132
DTA			1	.276**	-0.096
DTM				1	0.089
AR					1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

ANOVA TABLE for PH

SV	DF	SS	MS	F
replication	1	348.6736842	348.6736842	2.498605951
Treatment	189	25350.88421	134.1316625	0.961191467
Error	189	26374.44	139.547288	
Total	379	52073.99532		

For PH(Table **), we observed that $F_{cal.}=0.96<F_{(189,189)}(.05)=1.26$, so we can say that there is no significant difference between different genotype at 5% level of significance.

On the other hand, $F_{cal.}=0.96<F_{(189,189)}(.01)=1.39$, so the same trend is observed for 1% level of significance.

ANOVA TABLE for DTH

SV	DF	SS	MS	F
replication	1	20689.39	20689.39	198.096026
Treatment	189	12095.32632	63.99643553	0.612750781
Error	189	19739.39	104.4412141	
Total	379			

For DTH(table), we observed that $F_{cal.}=0.61<F_{(189,189)}(.05)=1.26$, so we can say that there is no significant difference between different genotype at 5% level of significance.
On the other hand, $F_{cal.}=0.61<F_{(189,189)}(.01)=1.39$, so the same trend is observed for 1% level of significance.

ANOVA TABLE for DTA

SV	DF	SS	MS	F
replication	1	41.12	41.12	4.600133964
Treatment	189	12400.05526	65.60875801	7.339996729
Error	189	1689.38	8.938526873	
Total	379			

For DTA (table), we observed that $F_{cal.}=7.33>F_{(189,189)}(.05)=1.26$, so we can say that there is significant difference between different genotype at 5% level of significance.
On the other hand, $F_{cal.}=7.33>F_{(189,189)}(.01)=1.39$, so we can say that there is significant difference between different genotype at 1% level of significance.

ANOVA TABLE for DTM

SV	DF	SS	MS	F
replication	1	62.41	62.41	1.358064356
Treatment	189	11347.68421	60.0406572	1.306495575
Error	189	8685.59	45.95549986	
Total	379			

For DTM (table), we observed that $F_{cal.}=1.30>F_{(189,189)}(.05)=1.26$, so we can say that there is significant difference between different genotype at 5% level of significance.
On the other hand, $F_{cal.}=1.30<F_{(189,189)}(.01)=1.39$, so we can say that there is no significant difference between different genotype at 1% level of significance.

ANOVA TABLE for AE

SV	DF	SS	MS
replication	1	0.07	0.07
Treatment	189	42.94182771	0.227205438
Error	189	15.09	0.079845284
Total	379		

For AE (table), we observed that $F_{cal.}=2.84>F_{(189,189)}(.05)=1.26$, so we can say that there is significant difference between different genotype at 5% level of significance.
On the other hand, $F_{cal.}=2.84>F_{(189,189)}(.01)=1.39$, so the same trend is observed for 1% level of significance.

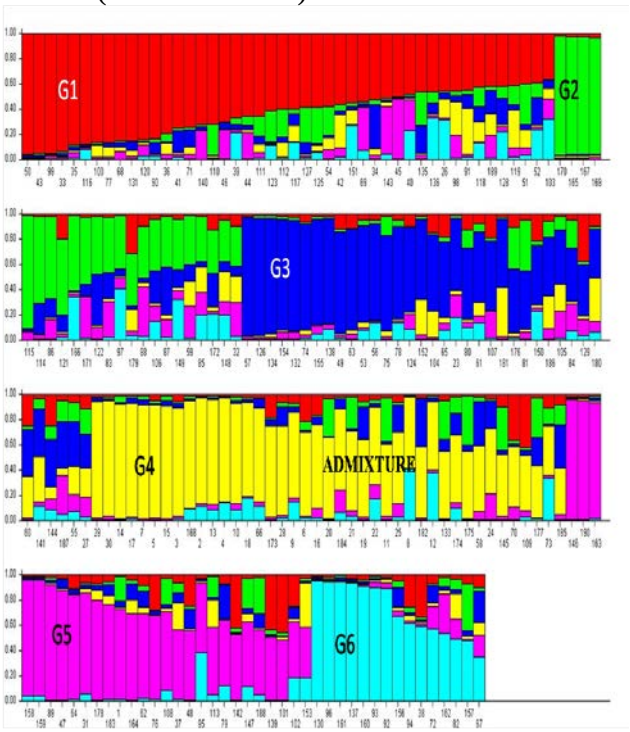
Marker trait association for Anther extrusion

Structure analysis of association mapping panel- LnPD values were used to determine the actual number of genetically distinct sub-populations (**Evanno *et al.*, 2005**). The analysis suggested that the AM panel is structured and all the genotypes were clustered into six sub populations i.e. G1, G2, G3, G4, G5, and G6. The six sub-population comprised 5 (G1), 7 (G2), 12 (G3), 12 (G4), 10 (G5) and 9 (G6) genotypes; the remaining 135 genotypes showed admixture.
Genetic relatedness of 190 wheat genotypes determined using 42 SNP markers through STRUCTURE analysis. Numbers on the y-axis indicate the membership coefficient. The colour of the bar indicates the six sub-populations identified through the STRUCTURE program (G1=red, G2=green, G3=blue G4=yellow G5=pink G6=sky blue). Genotypes with a similar colour belong to the same group.

Marker trait associations identified through GLM and MLM

In association analysis based on mean values over environments, significant association ($p<0.01$) of different traits (AE, DTH, DTA, DTM). DTH was observed for a total of 181 DArT SNP marker loci following GLM {The R2 values for each of these 181 DArT SNP marker varied from 3.68 % to 9.43 %.} and out of 101 DArT SNP marker were mapped on 19 wheat chromosomes and the remaining 80 SNPs could not be mapped. and for a total of 92 DArT SNP marker loci following MLM. and significent marker in MLM located on 16 wheat chromosomes (The results of MTAs are summarized in Table). Together 273 MTAs were

identified with 180 common MTAs from both the models (GLM and MLM).



AE was observed for a total of 177 DArT SNP marker loci following GLM {The R2 values for each of these 177 DArT SNP marker varied from 3.65 % to 13.01 %.} and out of 102 DArT SNP marker were mapped on 16 wheat chromosomes and the remaining 75 SNPs could not be mapped. and for a total of 142 DArT SNP marker loci following MLM. and significent marker in MLM located on 14 wheat chromosomes (The results of MTAs are summarized in Table). Together 319 MTAs were identified with 284 common MTAs from both the models (GLM and MLM).

DTA was observed for a total of 178 DArT SNP marker loci following GLM {The R2 values for each of these 178 DArT SNP marker varied from 3.51 % to 9.55 %.} and out of 168 DArT SNP marker were mapped on 14 wheat chromosomes and the remaining 10 SNPs could not be mapped. and for a total of 118 DArT SNP marker loci following MLM. and significent marker in MLM located on 18 wheat chromosomes (The results of MTAs are summarized in Table). Together 296 MTAs were identified with 284 common MTAs from both the models (GLM and MLM).

Conclusion

The important MTAs involving main effect and epistatic QTLs identified during the present study may be further validated using post-GWAS or joint linkage and association mapping (JLAM; Gupta *et al.*, 2019; Gahlaut *et al.*, 2019) and used for MAS in wheat breeding programmes targeted towards yield improvement. The correlation study revealed that plot yield had strong positive association with days to heading, days to anthesis, days to maturity & anther extrusion. The association studies, indicated that grain yield of wheat can be improved by selecting genotypes having higher performances for the above characters. Development of crop cultivars that are tolerant to abiotic stress has been an active area of research, but so far has met with only a limited success in field level. Anthers extrusion is most imported quantitative trait. Wheat AE (Anther extrusion) phenotypes were classified three classes (low, moderate and high anther extrusion).

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