Indian J. Genet., 78(1): 26-35 (2018)
DOI: 10.5958/0975-6906.2018.00004.4

Multi-environmental evaluation of wheat genotypes for drought tolerance

Sundeep Kumar*, Jyoti Kumari, Ruchi Bansal, B. R. Kuri, D. Upadhyay, Ashutosh Srivastava, Bhakti Rana[1], Manoj K. Yadav[1], R. S. Sengar[1], Amit K. Singh and Rakesh Singh

ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi 110 012; [1]SVP University of Agriculture & Technology, Meerut 250 110

(Received: June 2017; Revised: December 2017; Accepted: January 2018)

Abstract

Drought is a major abiotic stress affecting wheat production worldwide. Present study was conducted to identify drought tolerant wheat lines. In this study, field screening was done in multi-environment for four years followed by validation at molecular level for identifying stable drought tolerant wheat genotypes. In field screening, based on eleven quantitative traits including drought susceptibility index (DSI) under irrigated and non-irrigated conditions in two successive years, 44 wheat genotypes were selected from an initial set of 177 genotypes. These selected lines were further screened for two more years against drought stress. Stability analysis and AMMI biplot was also performed to analyze the stable performance of genotypes across the environments and years. The studied genotypes were also evaluated for the presence of six drought-linked molecular markers. Based on drought susceptibility index, other physiological parameters and molecular analysis, the genotypes namely, ET127225, ET127230, EC531185, ET127236, ET127267 and ET127269 were found to be potential genetic resources for drought tolerance, which can be further used in wheat improvement programme.

Key words: Drought stress, drought susceptibility index (DSI), molecular markers, multi-environmental trials, wheat genotypes

Introduction

Drought is one of the most devastating phenomenon, which occurs in all the climatic regions leading to the great losses in agriculture. There are projections that drought events may intensify in future making farming exceedingly challenging for the farmers. Wheat (*Triticum aestivum* L.) is the major staple crop satisfying hunger globally. Wheat production may decline substantially in China, India and Russia due to climate variability (Knox et al. 2012). Yield losses due to drought depend on the growth stage and severity of stress (Daryanto et al. 2016). Breeding for drought tolerance using novel genetic resources is the most viable strategy to cope with the changing climatic conditions (Mwadzingeni et al. 2016). However, due to limited availability of resistance sources, progress in breeding drought tolerant cultivars is not satisfactory. Drought tolerance is a complicated trait, which is controlled by polygenes and their expression is influenced by different environmental elements. It slows down the process of selection of drought tolerant genotypes. Selection based on drought adaptive traits along with the yield and its components may improve yield under target environment (Blum 2010; Monneveux et al. 2012; Passioura 2012). The yield components have been extensively used for screening against drought tolerance (Mwadzingeni et al. 2016). Days to heading and days to maturity also play an important role under terminal drought stress (Lopes et al. 2012).

Genotype and environment interaction studies are important for yield traits as the interaction plays a significant role in the expression of different genotypes in different environments. Previous study with wheat genotypes has shown that genotype-location, genotype-year and genotype-location-year interactions were highly significant for all the studied traits in wheat genotypes (Shah et al. 2009). Stability of wheat genotypes under different environments has also been studied (Aycicek et al. 2006; Akcura et al. 2011). The most frequently used technique for genotype stability estimation was proposed by Eberhart and Russell

*Corresponding author's e-mail: Sundeep.Kumar@icar.gov.in
Published by the Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com; www.isgpb.com

(1966). In addition, additive main effects and multiplicative interaction (AMMI) analysis is another approach, which combines both the ANOVA (with additive parameters) and Principal Component Analysis (with multiplicative parameters) into a single analysis (Zobel et al. 1988; Gauch et al. 1992; Gauch et al. 1996). It is also an effective tool to diagnose genotype environment interaction patterns graphically.

Molecular approaches can be an effective approach to further characterize genotypes for the presence of different drought tolerance genes/QTLs. The present study was therefore, conducted to evaluate a large number of genotypes for drought tolerance under irrigated and non irrigated conditions for two years. All the selected genotypes from an initial set of lines were further validated for the presence of drought tolerance QTLs/genes based on linked markers to identify the most promising drought tolerant genotypes.

Materials and methods

Plant materials and growth conditions

Initially, a set of 177 genotypes from East Gangetic Plain Sown Nursery (EGPSN) were evaluated for 11 different morpho-physiological traits *viz.,* germination percentage (GP), seedling survival (SS), days to 50% heading (DH), number of productive tillers (NT), plant height (PH) and days to maturity (DM), spike length (SL), the number of spikelets per spike (SLS), numbers of grains per spike (GS), thousand grain weight (TGW) and grain yield per m^2 (GY) under irrigated and non-irrigated conditions consecutively for two years 2007-08 and 2008-09 at SVP University of Agriculture & Technology, Meerut (latitude 28° 592 N, longitude 77° 422E, altitude 225m above mean sea level, maximum temperature 36.4° C and 37.3° C, annual rainfall 240 mm & 437.40 mm, soil pH 7.9). Based on two years trials, 44 genotypes were selected. Seeds of these genotypes were deposited in National Genebank and ET (Exotic Trials) number were allotted. These 44 selected genotypes were further evaluated for two more years at ICAR-National Bureau of Plant Genetic Resources, New Delhi (latitude 28° 352 N, longitude 70° 182 E, altitude of 226 m above mean sea level, maximum temperature 35.6° C and 35.7° C, annual rainfall 1056 mm & 604.20 mm, soil pH 7.5) during 2010-2011 and 2011-2012 under irrigated and non-irrigated conditions. The experiment was conducted in randomized complete block design (RCBD) with three replications. The genotypes were planted manually with gross plot size of 0.46 x 2.5 m with rows at 25 cm apart (4rows) and plant to plant distance of 10 cm. C306 (drought tolerant cultivar) and HUW206 (drought susceptible cultivar) were used as checks in trials. All the standard agronomic practices were followed to raise the crop. For treatment (drought), crop was irrigated only at crown root initiation stage and thereafter crop was raised under non-irrigated conditions, while under control condition, crop was irrigated as per the requirement of the crop. Data was recorded for all the 11 traits as mentioned above.

Molecular analysis

All the selected 44 genotypes were genotyped using six markers linked to drought tolerance. Markers linked to different physiological traits (chlorophyll content, photosystem II efficiency and leaf temperature) and yield were used for genotyping. The details of the primers used in the experiment are presented in Table 1. Total DNA was isolated from leaves and purified following the protocol of Saghai-Maroof et al. (1984), a CTAB based protocol. The amplification was performed in a 20μl reaction mixture consisting of 10x buffer with $MgCl_2$, 0.2 mM each dNTP, 0.25mM primer, 5U of *Taq* polymerase and 50ng genomic DNA. The Applied Biosystem 96 well thermal cycler, was programmed for: 4 minutes at 94°C, followed by 35, 40 or 45 cycles, each consisting of: 1 minute at 94°C, 45 sec. at 50°C to 60°C (according to the primer), 45 sec. at 72°C and a final extension of 7 minutes at 72°C. PCR products were separated by electrophoresis on 3% Metaphor agarose gel in 1x TAE buffer and images were recorded by gel documentation system (Alpha Imager, HP, Protein Simple Santa Clara, CA, USA).

Statistical analysis

The experimental design was a randomized complete block with a split-split plot treatment structure. Year and location combination was the main plot factor, irrigation was the sub plot factor and genotypes were considered as the split–split plot factor. Analysis of variance was performed using the GLM procedure in SAS (Version 9.3, SAS Institute). Environment, block, level of irrigation and genotype were used as class variables. For stability analysis, year and irrigation combination were considered together as eight environments. Stability analysis was performed following the model of Eberhart and Russell (1966). The sum of square due to G × E were portioned into individual genotypes (x-i), regression of environmental

Table 1. Description of the SSR markers used in this study

S.No.	Marker	Forward Primer	Reverse Primer	Ch. Location	Ps	Related Traits	reference
1.	GWM-169	5' ACCACTGCAGAGAACACATACG 3'	5' GTGCTCTGCTCTAAGTGTGGG 3'	6A, 1D	195 bp	FV/FM,CHL,LT	Kumar et al. 2012
2.	GWM-369	5' CTGCAGGCCATGATGATG 3'	5' ACCGTGGGTGTTGTGAGC 3'	3A	210 bp	FV/FM,CHL,LT	Kumar et al. 2012
3.	GWM-427	5' AAACTTAGAACTGTAATTTCAGA 3'	5' AGTGTGTTCATTTGACAGTT 3'	6A, 1D	205 bp	FV/FM,CHL,LT	Kumar et al. 2012
4.	GWM-484	5' ACATCGCTCTTCACAAACCC 3'	5' AGTTCCGGTCATGGCTAGG 3'	2D	210 bp	GFD and GY	Dodig et al., 2012a
5.	GWM-566	5' TCTGTCTACCCATGGGATTTG 3'	5' CTGGCTTCGAGGTAAGCAAC 3'	3B	140 bp	related to xanthophyll	Watson, 2008
6.	Barc-101	5' GCTCCTCTCACGATCACGCAAAG 3	5' GCGAGTCGATCACACTATGAGCCAATG 3'	3B	110bp	LT	Kumar et al. 2012

Where, Ch.= Chromosome; PS = Product size; FV/FM = Quantum efficiency of photosystem II; CHL= Chlorophyll; LT= Leaf temperature; GFD = Grain Filling Duration; GY = Grain Yield

means (bi) and deviation from regression (S^2d). The regression coefficients (bi) and mean square deviation from regression (S^2d) were used to define genotype stability i.e. suitability of a genotype for general cultivation over a wide range of environment. The environmental mean was the mean of all genotypes in each environment. The pooled error was used to test the hypothesis that the mean square deviation did not differ significantly from 0 at 0.05 and 0.01% probability levels. The t-test employing the standard error of regression coefficient against the hypothesis that it did not differ from 1.0 was performed. It was assumed that genotype effects were fixed and year effects were random. Further, AMMI model, which combines ANOVA with principal component analysis (PCA), was used to study the agronomic nature of genotype environment interactions using Proc IML procedures of SAS 9.3. Plots were prepared using mean and first two principal component (PC) scores as described by Zobel et al. (1988).

Results and discussion

All the genotypes showed significant variation for all the traits studied in different years. Experiment was laid down in RCBD with a split plot treatment, hence, interaction of irrigation x treatments, treatments x replication and year x irrigation x treatments were also analysed. The source of variation due to replication x years and irrigation was also significant for all the recorded traits except DM and GS, respectively. Treatment and replication interactions were significant for all the traits except SS, DH, DM and SL. However, in case of treatments, treatment x irrigation, treatment x irrigation x year, significant interaction was observed (Supplementary Table S1).

Correlation among the traits under irrigated and non-irrigated conditions

Correlation coefficient was estimated between all the traits using Pearson correlation coefficient under irrigated and non-irrigated conditions. The correlation coefficient of yield was significantly and positively correlated with NT (r=0.42) and GS (r=0.33), whereas TGW was found positively correlated with DM (r=0.32), GS (r=0.30) and yield (r=0.35) under irrigated conditions. Although genotype-by-environment interactions for drought stress were significant, the presence of significant positive correlations of yield with NT (r=0.57) and TGW (r=0.35), whereas TGW was significantly correlated with NT (r=0.30), DH (0.40) and DM (r=0.39) reflecting the accuracy and reproducibility of experimental conditions and of the scoring method used for drought stress evaluation. Mwadzingeni et al. (2016) reported strong positive correlations of productive tiller number with GY under both stressed and optimum conditions. Grain yield under stress was highly correlated with NT, with moderately high correlations with PH, GS, and TGW under stress. On the other hand, under optimum conditions, grain yield was highly and significantly correlated with all yield components except TGW which showed moderate correlation. Moderate to high positive and significant correlations ($r > 0.19$) of GY with NT, TGW and GS under both stressed and optimum conditions, imply the direct contribution of these yield components to yield and

should be considered as important target traits during selection, which is supported by the findings of Dodig et al. (2012b) and Sareen et al. (2014).

Per cent reduction and stress intensity for different traits

In general, reduction was observed for all the traits in non-irrigated conditions, except SS, DH and DM. Maximum reduction was recorded in yield (19.12%) followed by no. of tillers (17.6%) and 1000 grain weight (9.0%). It indicated that these traits are unstable in nature and thus highly influenced by environmental factors. Mean values of 46 genotypes including checks under four different environments were used to calculate stress intensity for different traits. The highest stress intensity was observed for yield followed by no. of tillers and 1000 grain weight, while, the lowest was observed in DH followed by DM and SS (Fig. 1).

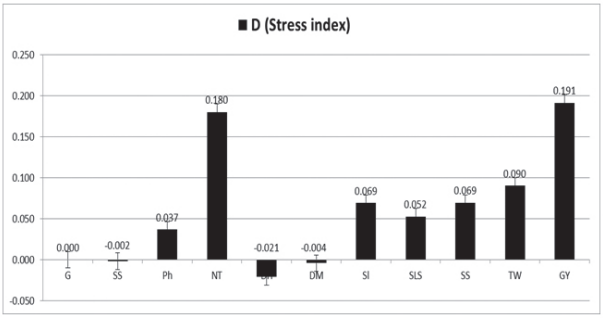

Fig. 1. Stress intensity of different traits for the combined data

Stability analysis for grain yield and 1000-grain weight

The genotypes and genotype x environment interaction component showed significant difference for GY and TGW. This indicated significant differences among the genotypes for linear response to environments and thus, behavior of the genotypes could be predicted over environments more precisely. Mean squares due to environment (linear) was found significant indicating differences between environment and their influence on genotypes for expression of these characters. This is in accordance with previous reports on rice, lentil and maize (Sawant et al. 2005; Panwar et al. 2008; Kumar et al. 2013; Jha et al. 2013). Hence, prediction of performance of genotypes based on stability parameters would be feasible and reliable. Significant linear component of G x E and non-linear components of G x E interaction were also noticed in previous studies (Gouri et al. 2008; Parry et al. 2008).

Eberhart and Russell (1966) defined a stable genotype as the one, which showed high mean yield, regression co-efficient (bi) around unity and deviation from regression near to zero. Accordingly, the mean and deviation from regression of each genotype was considered for stability analysis and linear regression was used for testing the varietal response. Genotypes with high mean, bi = 1 with non-significant δ^2di are suitable for general adaptation, i.e., suitable over all environmental conditions and they are considered as stable genotypes. Genotypes with high mean, bi > 1 with non-significant δ^2di are considered as below average in stability. Such genotypes tend to respond favourably to better environments but give poor yield in unfavourable environments. Hence, they are suitable for favourable environments. Genotypes with low mean, bi < 1 with non-significant δ^2di do not respond favourably to improved environmental conditions and hence, it could be regarded as specifically adapted to poor environments. Genotypes with any bi value with significant δ^2di are unstable. The genotypes C306, ET127224, ET127229, ET127233, IC598585, ET127239, EC531185, ET127257 and ET127269 had regression coefficient around 1, and among these genotypes, ET127224, ET127229, ET127233 and ET127239 had non-significant δ^2di and moderate grain yield hence could be considered for general adaptation to moisture limited as well as irrigated environments (Table 2; Fig. 2). Previous studies found that 'C306' was photosynthetically more stable than other genotypes under drought and high temperature stress and depended on current assimilate for grain growth (Al-Khatib and Paulsen, 1990; Yang et al. 2002). Similar results were observed in this experiment. Increased duration of synthesis of assimilates and the transport of assimilates to the kernel resulted in increased TGW. 'C306' and some of the drought-tolerant lines had high TGW and kernel number under water stressed condition, but most lines that had high Kernel number had relatively low TGW and vice versa. A kernel weight to kernel number compensation took place for efficient channelling of assimilates between the source and the sink (Davidonis et al. 2005; Shahinnia et al. 2005).

AMMI model is one of the most widely used statistical tools in the analysis of multi environmental trials. It can be used to understand and structure interactions between genotypes and environments. Main purpose of AMMI analysis is to understand the complex genotype x environment interaction, which includes delineating mega environments or identification of productive cultivars with wide

Table 2. Stability parameters of 46 wheat genotypes across 8 environments

Genotypes	Yield (g)			Genotypes	Yield (g)		
	Mean	b_i	S^2D_i		Mean	b_i	S^2D_i
C306	232.94	1.32	68.61*	ET127240	300.61	2.07	91.49*
HUW206	369.79	0.10	15.32	ET127241	343.47	0.07	73.32*
IC0143970	444.08	0.10	36.55	ET127242	227.94	0.10	79.33*
ET127182	532.12	0.13	15.13	ET127243	212.84	0.15	93.50*
ET127192	636.56	0.38	45.30	ET127244	485.77	0.13	97.24*
ET127195	234.92	0.61	26.70	ET127245	221.18	1.27	220.80**
ET127198	255.21	0.16	30.63	ET127246	436.05	0.07	198.22**
ET127221	367.46	0.08	25.74	ET127251	203.63	2.32	167.31**
ET127222	563.14	3.50	351.81**	ET127252	215.75	3.07	4918.21**
ET127223	312.17	0.11	68.79*	ET127253	272.32	2.36	10513.89**
ET127224	201.81	1.57	57.03	ET127254	234.72	0.35	242.78**
ET127225	296.55	0.09	66.16*	ET127256	331.54	4.42	31674.77**
ET127226	257.09	0.05	60.17	ET127257	618.92	1.27	27195.03**
ET127227	436.76	0.06	63.32	ET127261	246.09	2.12	519.45**
ET127228	342.66	3.24	268.59	ET127265	246.45	3.17	2400.40**
ET127229	255.79	0.96	140.73	ET127267	310.95	0.07	75.01*
ET127230	273.61	0.51	55.69	ET127269	294.43	1.05	5969.00**
ET127232	228.82	0.63	59.71	ET127270	281.75	3.085	10608.87**
ET127233	186.42	1.45	22.59	ET127273	261.74	0.53	771.39**
EC531185	429.38	0.60	43.53	ET127276	390.22	0.11	256.15**
ET127236	515.37	0.09	84.69	ET127278	360.75	0.39	103.08**
ET127238	593.87	0.14	285.22	ET127279	350.49	0.16	344.27**
ET127239	178.07	0.92	59.91	ET127280	301.11	0.56	69.05*

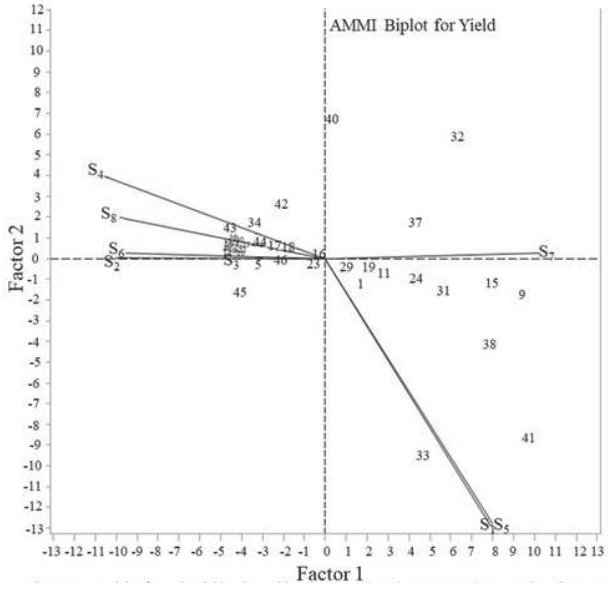

Fig. 2. AMMI Biplot for grain yield under multi-environments (S1-S8), S1: 2007-08 (Meerut-Irrigated), S2: (Meerut-drought), S3: 2008-09 (Meerut Irrigated, S4: 2008-09 (Meerut-drought), S5: 2010-11 (New Delhi-It), S6: 2010-11 (New Delhi-drought), S7: 2011-12 (New Delhi-Irrigated), S8: 2910-11 (New Delhi-drought)

adaptability, as well as delimit the agronomic zoning of cultivars with specific adaptability (Gauch et al. 2011; Gauch 2013). In the AMMI analysis, employing Gollob's test first two principal components (PC) explained 100% of the G X E variation PC 1 and PC 2 explained 72.96% and 27.03% for grain yield. The graphical method was employed by using two PC to investigate environmental variation and interpret the G x E interaction. The environments showed much variability in both main effects and interactions. Environments S_3 and S_5 were most discriminating

followed by S_7, S_4, S_2, S_6 and S_8 with equal discrimination. The environments (S_3 and S_5) and (S_2 and S_6) are more similar in performance that is predictable as both locations fall in the north western plain zone of India. Genotypes ET127253 and ET127270 were more suitable for high yielding environment S_3. Genotypes C306, ET127253, ET127225, ET127229, ET127232, ET127233, ET127239, ET127245, ET127254, EC531185, ET127270 and ET127278 were widely adapted as they were plotted near origin of the biplot (Table 2; Fig. 2). Thus, based on AMMI and Eberhart and Russell stability analysis widely adapted genotypes were ET127229, ET127233 and ET127239 yielding stable performance across the environments irrespective of irrigation regimes and locations.

Identification of promising genotypes under irrigated and non-irrigated environments

The genotypes were grouped into four categories based on the drought susceptibility index (DSI): Drought tolerant (DT DSI ~0.5), moderately drought tolerant (Mod-DT DSI ~0.5 - ~1.0), moderately drought susceptible (Mod-DS DSI ~1.0 - ~1.5) and drought susceptible (DS DSI ~1.5) with some modifications as proposed by Tiwari et al. (2014). Based on the DSI value for 1000 grain weight and grain yield m^{-2}, drought tolerant genotypes were identified (Fig. 3). Genotypes,

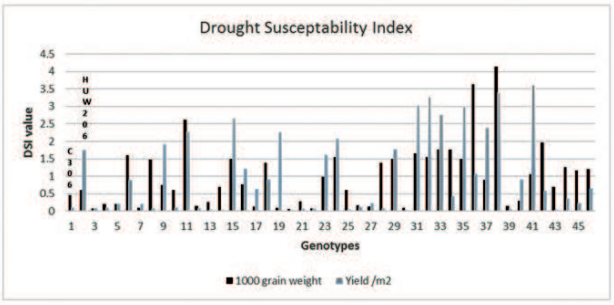

Fig. 3. Drought susceptibility index of 46 wheat genotypes for 1000-grain weight and yield/m^2

which showed DSI value less than 0.5 for 5-6 traits including yield/m^2 were considered drought tolerant. Based on this, ET127225, ET127230, EC531185, ET127236, ET127238, ET127246, ET127267 and ET127269 were considered drought tolerant. Genotypes ET127223, EC531185, ET127236 and ET127261 were highly drought tolerant and far better than standard check, C306, a known drought tolerant cultivar.

Table 3. Rank wise top ten promising genotypes for no. of grains per spike, 1000-grain weight and yield m^{-2} based on mean values under irrigated and non-irrigated conditions

No. of grains per spike (GS)				1000 grain weight (GW)				Yield per meter^2 (YM)			
Irrigated		Non-irrigated		Irrigated		Non-irrigated		Irrigated		Non-irrigated	
Geno.	Mean	Geno.	Mean	Gen	Mean	Geno.	Mean	Geno.	Mean	Geno.	Mean
ET127252	62.4^{a}	ET127252	62.7^{a}	ET127238	54.5^{a}	ET127238	54.1^{a}	ET127222	688.9^{a}	ET127192	623.3^{a}
ET127225	62.0^{ab}	ET127225	61.9^{ab}	ET127227	51.6^{b}	ET127192	49.5^{b}	ET127257	688.6^{a}	ET127238	590.6^{b}
ET127182	60.7^{bc}	ET127238	60.7^{b}	ET127257	51.5^{b}	IC0145970	48.6^{bc}	ET127192	649.9^{b}	ET127257	549.3^{c}
ET127238	60.4^{bc}	ET127232	59.1^{c}	ET127251	51.5^{b}	ET127243	48.4^{bc}	ET127238	597.1^{c}	ET127180	527.7^{d}
ET127192	59.4^{cd}	ET127227	52.4^{d}	ET127273	51.3^{b}	ET127227	48.3^{c}	ET127182	536.6^{d}	ET127236	512.5^{e}
ET127232	58.8^{d}	EC531185	52.4^{d}	ET127228	51.1^{bc}	ET127246	46.7^{d}	ET127236	518.3^{e}	ET127244	482.2^{f}
EC531185	52.7^{e}	ET127257	51.0^{e}	ET127192	50.4^{cd}	ET127241	46.5^{de}	ET127244	489.3^{f}	IC0145970	441.0^{g}
ET127227	52.1^{e}	ET127236	50.9^{e}	ET127252	50.3^{d}	ET127223	46.3^{def}	ET127256	464.0^{g}	ET127222	437.4^{g}
ET127273	51.9^{e}	ET127230	50.4^{ef}	ET127254	50.1^{d}	ET127242	45.8^{defg}	ET127228	458.9^{g}	ET127226	435.6^{g}
ET127241	51.8^{e}	ET127239	49.7^{ef}	ET127241	49.2^{e}	ET127229	45.5^{efg}	IC0145970	447.2^{h}	ET127246	435.0^{g}

Table 4. Details of 46 genotypes along with their pedigree and banding pattern produced by molecular markers associated with different drought tolerance traits

S.No.	Genotype No.	Pedigree	GWM-169 (220bp)	GWM-369 (210bp)	GWM-427 (180bp)	GWM-484 (180bp)	GWM-566 (150bp)	BARC-101 (80bp)
1.	C306	RGN/CSK 3//2* C591/3/C217/ N14// C281	+	+	-	+	+	+
2.	HUW 206	KAVKAZ/BUHO/KALYANSONA/BLUE BIRD	-	+	-	-	-	-
3.	IC0143970	SONALIKA (II54-368/An/3/Yt54/N10B//LR64 (II18427-4R-1M)	-	-	-	-	-	_
4.	ET127182	ELVIRA/CHIBIA	-	-	-	+	-	_
5.	ET127192	DUCULA/KAUZ//WBLL1	-	-	+	-	-	_
6.	ET127195	PICUS/3/KAUZ*2/BOW//KAUZ/4/TILHI	-	+	+	-	-	_
7.	ET127198	ALD/CEP75630//CEP75234/PTT219/3/BUC/ BJY/4/CBRD/5/TNMU/PF85487	-	-	+	-	-	_
8.	ET127221	SIRKKU/FINSI	-	+	+	+	+	+
9.	ET127222	FRET*2/4/SNI/TRAPSNI /TRAP 1/3/KAUZ*2/ TRAP//KAUZ	-	-	+	-	-	+
10.	ET127223	FRET2/TUKURU//FRET2	+	+	+	-	-	+
11.	ET127224	WBLL*2/4/SNI/TRAP= 1/3/KAUZ*2/TRAP//KAUZ	-	+	+	-	+	+
12.	ET127225	C80.1/3*BATAVIAL//2*WBLL1	+	+	-	-	+	+
13.	ET127226	CHBIA//PRLII/CM65531/3/FISCAL	-	-	-	-	+	+
14.	ET127227	KIRITATI//PRL/2*APASTOR	-	+	+	+	+	+
15.	ET127228	PFAU/WEAVER*2//KIRITATI	+	-	+	-	-	_
16.	ET127229	SERI/RAYON	-	-	+	+	-	_
17.	ET127230	CS/TH.CS//3*PVN/3/MIRLO/BUC/4/MILAN/5/TILHI	-	+	+	-	+	+
18.	ET127232	TOBA97/PASTOR	-	-	+	-	-	+
19.	ET127233	OASIS/SKAUZ//4*BCN*2/3/PASTOR	+	-	+	+	+	+
20.	KANCHAN	UP 301/C306 (VARIETY FROM BANGLADESH) (EC531185)	-	+	+	+	+	+
21.	ET127236	SW90.1057/3/KAUZ*2/YACO//KAUZ*2/YACO// KAUZ	-	+	+	-	+	+
22.	ET127238	SUN290B/6/CHIBIA/5/CNDO/R143/ENTE/MEXI-2/3/AE.SQUA(TAUS)/4/WE	-	+	+	-	-	+
23.	ET127239	QT8368*2/5/CPI/GEDIZ/3/GOO//JO69/CRA/4/ AE.SQUA(208)	-	+	-	-	-	+
24.	ET127240	PASTOR//SITE/MO/3/CHEN/AE.SQUA(TAUS)// BCN/4/T.TAU.83.2.36/5/QTS	-	-	-	-	-	_
25.	ET127241	SHARP/3/PRL/SARA//TSI/VEE#5/5/VEE/LIRA/ BOW/3/BCN/4/KAUZ	+	-	+	-	-	+
26.	ET127242	CROC-1/AE.SQUA(205)//KAUZ/3/ENEIDA/4/ PSN/BOW//MILAN	-	+	-	-	-	_
27.	ET127243	WBLL 4//OAX 93.24.35/WBLL 1	-	-	-	+	-	_
28.	ET127244	MUNIA/CHIO/3/PFAU/BOW//VEE#9/4/CHEN/ AE.SQUA(TAUS)//BCN/5/BAB	-	-	-	-	+	_
29.	ET127245	WAXWING*2/BRAMBLING-1	-	-	-	-	+	_
30	ET127246	WAXWING*2/BRAMBLING-2	-	+	+	-	-	_

31.	ET127251	WHEAR/KIRITATI/3/C80.1/3*BATAVIA//2*WB	-	-	+	+	+	_
32.	ET127252	WHAER/4/SNI/TRAP#1/3/KAUZ*2/TRAP// KAUZ/5/C80.1/3*BATAVIA//2*WB-1	-	+	+	+	+	_
33.	ET127253	WHAER/4/SNI/TRAP#1/3/KAUZ*2/TRAP// KAUZ/5/C80.1/3*BATAVIA//2*WB-2	-	-	+	+	-	_
34.	ET127254	WHEAR//2*PRL/2*PASTOR	-	-	+	-	-	_
35.	ET127256	MILAN/S 87230//BABAX	-	-	+	+	+	+
36.	ET127257	SABUF/7/ALTAR84/AE.SQUA9224)//YACO/6/ CROC-1/AE.SQUA(205)/5/BR	-	-	+	-	-	+
37.	ET127261	KIRITATI/4/SERI.1B*2/3/KAUZ*2/BOW//KAUZ	-	+	-	-	-	+
38.	ET127265	WEAVER/TSC//WEAVER/3/WEAVER/4/ 2*PRL/2*PASTOR	-	-	-	-	-	+
39.	ET127267	ATTILA*2/STAR*2//VIVITSI	-	-	-	+	+	+
40.	ET127269	KIRITATI//SERI/RAYON	+	+	+	+	-	+
41.	ET127270	ATTILA*2/STAR*2/4/SNI/TRAP#1/3/KAUZ*2/ TRAP//KAUZ	+	+	+	+	+	+
42.	ET127273	BAW 969)/SHATABDI-1	-	+	-	-	+	+
43.	ET127276	BAW 969)/SHATABDI-2	-	+	+	-	+	_
44.	ET127278	BAW 923/BAW 1008-1	-	-	+	-	-	_
45.	ET127279	BAW 923/BAW 1008-2	-	+	-	-	-	+
46.	ET127280	BAW 923/BAW 1008-3	+	+	-	+	-	_

Note: + for presences and – for absence of band.

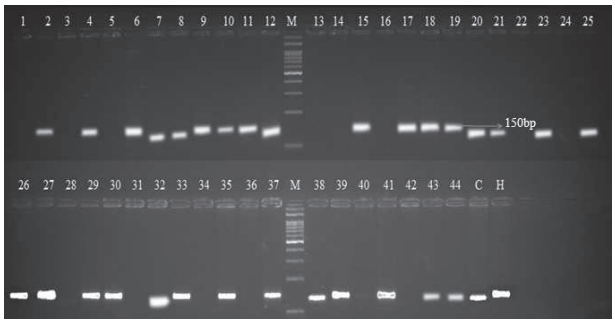

Fig. 4. PCR profile of 46 wheat genotypes with SSR marker GWM-566. Sample No. 1- 44 are the selected wheat genotypes; C=C306, H=HUW206. M is 100bp DNA Ladder

Trait wise promising genotypes under irrigated conditions were ET127257 (grain yield, 1000 grain weight), ET127182 (grain yield, grains per spike), ET127228 (grain yield, 1000 grain weight). While, under non-irrigated conditions in ET127192, ET127238, IC0145970 and ET127227 (grain yield and 1000 grain weight), ET127257 (grain yield and grains per spike) and ET127246 (grain yield and 1000 grain weight). Genotypes ET127192 and ET127238 were found promising 1000-grain weight and grain yield under irrigated as well as non-irrigated conditions (Table 3).

Molecular marker screening for drought tolerance

Drought tolerance being a complex trait is difficult to phenotype under field conditions. Marker assisted selection using drought tolerance QTLs or candidate genes based molecular markers can facilitate tracking of drought tolerance & QTLs and thereby accelerate selection of drought tolerant genotypes in wheat breeding program. Moreover, such markers need to be exploited to screen germplasm collection for selecting potential drought tolerant genotypes. Many studies have reported molecular markers linked to various drought tolerance traits are available in wheat (Wei et al. 2009; Kumar et al. 2012; Mohammadi et al. 2008; Nezhadahmadi et al. 2013). Forty four selected genotypes were screened using six molecular markers associated to drought tolerant traits *viz*., photosystem II efficiency (F_V/F_M), Chlorophyll content, leaf temperature, xanthophyll content, membrane stability, GFD (grain filling duration) and grain yield (Table 1). Genotypes with higher chlorophyll fluorescence, chlorophyll content and cooler canopy tend to be more tolerant to drought conditions (Pradhan et al., 2012). These drought tolerance associated markers can be used for selecting drought tolerant genotypes in combination with other markers and physiological parameters like drought susceptibility index (DSI).

Seven wheat genotypes namely ET127221, ET127225, ET127227, EC531185, ET127236, ET127267 and ET127269 produced predicted size amplicons with four or more drought-linked markers (Table 4; Fig. 4). These genotypes were also found drought tolerant based on drought susceptibility index (DSI). Similar results were reported by Kirigwi et al. (2007), who observed the association of molecular markers with the quantitative traits in wheat genotypes. Nevertheless, three genotypes IC0145970, ET127192 and ET127254 showed DSI (yield) <0.5, while none of the selected drought tolerant QTL was present in these genotypes. This suggests that the drought tolerance in these genotypes might be due to the presence of other tolerance genes/QTLs, which have not been included in the present study.

Authors' contribution

Conceptualization of research (SK, JK); Designing of the experiments (SK, BR, MKY, RSS); Contribution of experimental materials (SK, MKY); Execution of field/lab experiments and data collection (SK, BR, JK, BBK, AS, DU); Analysis of data and interpretation (JK, RB, BRK, AKS, RS); Preparation of manuscript (SK, JK, RSS, RB, DU, AS).

Declaration

The authors declare no conflict of interest.

References

Akçura M., Taner S. and Kaya Y. 2011. Evaluation of bread wheat genotypes under irrigated multi-environment conditions using GGE biplot analyses. Agriculture, **98**: 35-40.

Aycicek M. and Yildirim T. 2006. Heritability of yield and some yield components in bread wheat (*Triticum aestivum* L.) genotypes. Bangladesh J. Bot., **35**: 17-22.

Al-Khatib K. and Paulsen G. M. 1990. Photosynthesis and productivity during high-temperature stress of wheat genotypes from major world regions. Crop Sci., **30**: 1127-1132.

Blum A. 2010. Plant Breeding for Water-Limited Environments. London: Springer.

Daryanto S., Lixin W. and Pierre-André J. 2016. Global synthesis of drought effects on maize and wheat production. PLOS ONE, **11**(5): e0156362.

Dodig D., Zoric M., Kobiljski B., Savic J., Kandic V., Quarrie S., and Barnes J. 2012a. Genmetic and association mapping study of wheat agronomic traits under contrasting water regimes. International J. Mol. Sci., **13**(5): 6167-6188.

Dodig D., Zorić M., Kandic V., Perović D., Šurlan Momirović G. 2012b. Comparison of responses to drought stress of 100 wheat genotypes and landraces to identify opportunities for improving wheat drought resistance. Plant Breed., **131**: 369-379.

Davidonis G. H., Richard O. A., Ingber B. F., Meredith W. R. and Heitholt J. J. 2005. The influence of cotton seed weight on fibers per seed and fiber property uniformity. J. New Seeds, **7**:1-13.

Eberhart S. A. and Russell W. A. 1966. Stability parameters for comparing varieties. Crop Sci., **6**: 36-40.

Gauch H. G. 1992. Statistical Analysis of Regional Trials, AMMI Analysis of Factorial Designs. Elsevier, Amsterdam, the Netherlands, pp. 278.

Gauch H. G. and Zobel R. W. 1996. AMMI analysis of yield trials. In Kang MS, Gauch Jr HG. (ed.) Genotype by environment interaction. CRC Press, Boca Raton, FL, pp. 85-122.

Gauch H.G. et al. 2011. Two new strategies for detecting and understanding QTL x environment interactions. Crop Sci.,**51**: 96-113.

Gauch H.G. 2013. A simple protocol for AMMI analysis of yield trials. Crop Sci., **53**: 1860-1869.

Gouri S. V., Ansari N. A. and Ilias A. M. 2008. Stability analysis using thermo-sensitive genic male syerility (TGMS) system in rice (*Oryza sativa* L.). Res. on Crops, **9**: 141-146.

Jha S. K., Singh N. K., Kumar R. Arun, Agrawal P. K., Bhatt J. C., Guleria S. K., Lone A. A., Sudan R.S., Singh K.P. and Mahajan V. 2013. Additive main effects and multiplicative interaction analysis for grain yield of short duration maize hybrids in North-Western Himalayas. Indian J. Genet., **73**(1): 29-35.

Kirigwi F. M., Van G. M., Brown-Guedira G., Gill B. S., Paulsen G. M. and Fritz A. K.2007. Markers associated with a QTL for grain yield in wheat under drought. Mol. Breed., **20**: 401-413.

Knox J., Hess T., Daccache A. and Wheeler T. 2012. Climate change impacts on crop productivity in Africa and South Asia. Environ. Res. Lett., **7**: 1-8.

Kumar H., Dikshit H. K., Singh A. M., Singh D., Kumari Jyoti., Singh A. and Kumar D. 2013. Characterization of elite lentil genotypes for seed iron and zinc concentration and genotypes x environment interaction studies. Indian J. Genet., **73**(2): 169-176.

Kumar S., Sehgal S. K., Kumar U., Prasad P. V. V., Joshi A. K. and Gill B. S. 2012. Genomic characterization of drought related traits in spring wheat. Euphytica, **186**: 265-276.

Lopes M., Reynolds M., Jalal-Kamali M., Moussa M., Feltaous Y. and Tahir I. 2012. The yield correlations of selectable physiological traits in a population of advanced spring wheat lines grown in warm and

drought environments. Field Crops Res., **128**: 129-136.

Mohammadi A., Tabatabaeefar A., Shahan S., Rafiee S. and Keyhani A. 2008. Energy use and economical analysis of potato production in Iran a case study: Ardabil province. Energy Convers. Manage., **49**: 3566-3570.

Monneveux P., Jing R. and Misra S. 2012. Phenotyping wheat for adaptation to drought using physiological traits. Front Physiol., **3**: 429.

Mwadzingeni L., Hussein S., Samson T., Toi J. and Tsilo 2016. Screening of Bread Wheat Genotypes for Drought Tolerance Using Phenotypic and Proline Analyses. Front. Plant Sci., **7**: 1276.

Nezhadahmadi A., Prodhan Z.H. and Faruq G. 2013. Drought tolerance in wheat. The Scientific World J., 1-12.

Panwar L. L., Joshi V. N. and Mashiat A. 2008. Genotype x environment interaction in scented rice. *Oryza*, **45**: 103-109.

Parry G. A., Shikari A. B., Manzoor G. A. and Najib A. S. 2008. Stability in elite rice genotypes under high altitude environments of Kashmir valley. Res. on Crops, **9**: 131-138.

Passioura J. 2012. Phenotyping for drought tolerance in grain crops: when is it useful to breeders? Funct. Plant Biol., **39**: 851-859.

Pradhan G. P., Prasad P. V. V., Fritz A. K., Kirkham M. B. and Gill B. S. 2012. Response of Aegilops species to drought stress during reproductive stages of development. Funct. Plant Biol., **39**: 51-59.

Saghai-Maroof M. A., Soliman K. M., Jorgensen R. A. and Allard R. W. 1984. Ribosomal DNA spacer-length polymorphism in barley: mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci., **81**: 8014-8019.

Sawant D. S., Kunkerkar R. L., Shetye V. N. and Shirdhankar M. M. 2005. Stability assessment in late duration rice hybrids. In: National seminar on "Rice and Rice Based Systems for Sustainable Productivity, Extended summaries, ICAR Research Complex for Goa", 18-19th October, pp. 75-76.

Sareen S., Tyagi B. S., Sarial A. K., Tiwari V. and Sharma I. 2014. Trait analysis, diversity, and genotype x environment interaction in some wheat landraces evaluated under drought and heat stress conditions. Chil. J. Agric. Res., **74:** 135-142.

Shahinnia F., Rezai A. M. and Sayed Tabatabaei B. E. 2005. Variation and path coefficient analysis of important agronomic traits in two- and six-rowed recombinant inbred lines of barley (*Hordeum vulgare* L.). Czech. J. Genet. Plant Breed., **41**: 246-250.

Shah T. 2009. Taming the anarchy: groundwater governance in South Asia. Resources for the Future, Washington, DC, and International Water Management Institute, Colombo, Sri Lanka.

Tiwari A. S., Sourabh P. and Verulkar S. B. 2014. Screening of F_3 Segregating population for drought tolerance through drought susceptibility index in rice. Plant Archives, **14**(1): 23-28.

Wei B., Jing R. L ., Wang C. S., Chen J. B., Mao X. G., Chang X. P. and Jia J. Z. 2009. *Dreb1* genes in wheat (*Triticum aestivum* L.): Development of functional markers and gene mapping based on SNPs. Mol. Breed., **23**: 13-22.

Yang J., Peng S., Zhang Z., Wang Z., Visperas R. M. and Zhu Q. 2002. Grain and dry matter yields and partitioning of assimilates in japonica/indica hybrid rice. Crop Sci., **42**: 766-772.

Zobel R. W., Wright M. J. and Gauch J. H. G. 1988. Statistical analysis of a yield trial. Agron. J., **80**: 388-393.

Supplementary Table S1. Analysis of variance for eleven different traits compared under irrigated and non-irrigated conditions

Source	DF	GP	SS	PH	NT	DH	DM	SL	SLS	GS	GW	YM
Year	3	197.38**	364.32**	2946.38**	15.77**	392.53**	798.13**	14.25**	49.81**	218.66**	1457.46**	13639.81**
Rep(Yr)	6	3.85**	11.56**	24.17**	11.02**	2.02**	0.47	63.61**	67.92**	140.38**	25.24**	319.21**
Irrig	1	0.12	7.68**	3139.35**	740.77**	702.66**	72.80**	142.93**	1116.47**	2995.80**	4778.70**	1361456.76**
Trt	45	21.77**	41.07**	1759.76**	71.96**	91.06**	79.43**	45.97**	395.33**	1169.17**	489.08**	355285.72**
Irrig*Trt	45	0.36**	0.65**	176.17**	26.07**	4.59**	4.90**	4.67**	58.45**	251.11**	92.40**	44481.12**
Trt*Rep	90	0.10**	0.21	3.16**	1.00**	0.43	0.82	0.43	2.07*	5.17**	1.87*	139.68**
Yr*Irrig*Trt	273	0.38**	0.49**	44.58**	1.64**	5.19**	3.13**	0.89**	6.16**	21.83**	6.57**	6481.85**
R-Square		0.99	0.95	0.99	0.97	0.98	0.96	0.96	0.97	0.97	0.98	1.00
CV		0.25	0.62	1.55	8.03	0.83	0.77	7.54	3.38	4.04	2.85	2.71
Root MSE		0.24	0.58	1.40	0.67	0.65	0.94	0.76	1.26	1.86	1.26	9.00
Mean		95.12	93.55	90.55	8.29	77.71	121.54	10.05	37.39	45.96	44.09	332.44

Where, DF=Degree of freedom; GP=Germination percentage; SS=Seedling survival; PH=Plant height; NT=Number of tillers; DH=Days to heading; DM=Days to maturity; SL=Spike length; SLS=Spikelets per spike; GS=Grains per spike; TGW=1000 grain weight; YM=yield/m^2