



Evaluation of Drought Tolerance in Emmer Based Wheat Double Haploid Lines using Surrogate Traits and Stress Tolerance Indices

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Abstract

Wheat is one of the major cereals and staple food cultivated worldwide, affected by severe environmental and climate changes. Global warming has led to significant variations in wheat production, accounting for losses up to 6.4% for each 1°C rise in temperature. Recurrent droughts in cultivable areas are anticipated to increase the risk of wheat yield loss by almost 12% by the end of the 21st century. The present study was undertaken to identify component traits contributing to drought tolerance and to evaluate wheat doubled haploid (DH) lines for drought tolerance using various stress tolerance indices. Fifty-three wheat DH lines and 11 check varieties were evaluated for grain yield, agronomic, physiological traits and drought tolerance indices under *rainfed* and restricted irrigation conditions in the 2019-20 cropping season. The results of ANOVA revealed that various yield contributing, agronomic and physiological traits showed highly significant genotypic variation. A significant positive correlation was observed between grain yield under *rainfed* (Y_s) and restricted irrigation (Y_p) conditions and geometric mean productivity (GMP), mean productivity (MP), harmonic mean (HM), stress tolerance index (STI) and yield index (YI). Principal component analysis (PCA) showed a significant positive correlation between stress susceptibility index (SSI) and tolerance (TOL). Based on PCA and cluster analysis, the test entries like DH 989 and DH 554, along with check cultivars NI 5439, NIAW 1415, DPW 621-50, were more stable in the *rainfed* environment while DH 807, DH 430, DH 435, DH 417 and DH 431 were highly adapted to restricted irrigation conditions. DH 66, DH 227, DH 931, DH 62, DH 942, DH 875, DH 134 and DH 179 were identified as susceptible genotypes. Cluster analysis also classified the genotypes into four groups,



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i.e., resistant, susceptible, tolerant and moderately tolerant to drought conditions. As per our study, few genotypes susceptible to drought exhibited yield reduction while the tolerant genotypes were stable, suggesting their genetic variability and utilization in a breeding program.

Introduction

Bread wheat (*Triticum aestivum* L.) is an annual crop from the Poaceae family. It is a staple food in many countries. It supplies nutrients to nearly 2.5 billion people constituting 36% of the global population. Globally, wheat is cultivated over 217 million hectares with a production of 752 million tonnes and productivity of 3.5 tonnes/ha.¹ According to the fourth advance estimate 2021-22, India stands second in wheat production with 106.84 million tonnes² from 30 million hectares. However, these wheat production trends need to be maintained despite several biotic and abiotic factors, of which drought is one of the major constraints to wheat productivity.³ The intensity of drought has increased due to global warming and climate change, which has negatively impacted crop growth and yield resulting in the decline of wheat production.⁴ More than one million people are facing the food crisis, which is expected to double by 2050.⁵ Mitra (2001)⁶ emphasized drought as a matter of great concern for the breeders, resulting in yield loss due to dwindling water resources and increasing drought intensity. This has necessitated developing and identifying the genotypes with sustainable yield potential under drought conditions. Nouri *et al.* (2011)⁷ recognized that the relative yield performance of genotypes in drought-stressed and favourable environments helps to identify the desirable genotypes under *rainfed* conditions. Many researchers trust in selecting genotypes under both favourable and unfavourable conditions.^{7,8,9,10,11,12} Identifying component traits contributing to drought tolerance is an inevitable step in the wheat improvement program. Many surrogate traits for drought tolerance have been identified in wheat, which includes traits related to vegetative growth, agronomic parameters, water use efficiency, and photoprotection. However, their associations with yield are poorly understood. This may be due to the fact that wheat is grown under very different climatic conditions across the world and hence faces different drought scenarios.¹³ Therefore, there is a need to identify location-specific traits for drought tolerance. Various drought tolerance indices have

been identified based on the potential yield (Y_p) under restricted irrigation and yield under water stress conditions (Y_s). The stress susceptibility index (SSI) was used by Fischer and Maurer (1978)¹⁴ to measure yield stability, which caused the changes in potential and actual yields in variable environments. SSI was also used to examine drought-resistant genotypes of wheat by researchers like Clarke *et al.*, 1992¹⁵ and Guttieri *et al.*, 2001.¹⁶ According to Guttieri *et al.*, 2001¹⁶ genotypes with SSI less than 1 are more resistant to drought stress conditions and vice-versa. According to Fernandez (1992),¹⁷ the Stress Tolerance Index (STI) is used to find the genotypes with high yield under stress and non-stress environments. Rosielle and Hamblin (1981)¹⁸ stated that stress tolerance (TOL) is the difference between stress and irrigated environments, and mean productivity (MP) is the average yield of genotypes under stress and non-stress conditions. Geometric mean productivity (GMP) describes the relative performance. The yield index (YI) (Gavuzzi *et al.*, 1997)¹⁹ and yield stability index (YSI) suggested by Bouslama and Schapaugh (1984)²⁰ are the other yield-based estimates that evaluate the stability of genotypes in both stress and non-stress conditions. Many researchers have used them to screen drought-tolerant wheat genotypes. A significantly positive correlation between grain yield and GMP, MP and STI under stress and non-stress conditions signifying their effectiveness for screening drought-tolerant bread wheat genotypes, was reported by Sio-Se Mardeh *et al.* (2006),¹⁰ Safarnejad (2004),²¹ Sanjari *et al.* (2006),²² Farshadfar and Sutka (2003)²³ and Geravandi *et al.* (2010).²⁴ Hessadi (2006)²⁵ reported the suitability of GMP, MP, harmonic mean (HM) and STI indices in selecting high-yielding barley cultivars under drought conditions. Emmer wheat is considered to have drought tolerance. So the present study was aimed to evaluate the drought tolerance in emmer based hexaploid wheat to predict drought tolerance traits and suitable genotypes tolerant to drought stress.

Materials and Methods

In the present study, 64 wheat genotypes, including 53 emmer-based doubled haploid lines and 11 Indian cultivars were used in the experiment to assess their drought tolerance. Eleven cultivars used as checks for *rainfed*, restricted irrigation and irrigated conditions in peninsular and central zones of India, were used in this trial. A trial comprising of 3 replications and two treatments, viz., *rainfed* (RF) and restricted irrigation (RI), was constituted at Agharkar Research Institute's experimental farm located at Hol, Tal-Baramati, Dist-Pune, in the Peninsular zone of India (Latitude-18.04° N, Longitude -74.21° E, altitude-548.6m) in 2019-20 cropping season. The trial was sown in Mid-November with lattice design. The gross plot size was 3m x 3 rows, 0.23m apart (2.07 sq. m.), and the net plot size was 2 m x 3 rows, 0.23 cm apart (1.38 sq.m.). The recommended dose of fertilizers was used as prescribed for *rainfed* and restricted irrigation. The field was irrigated immediately after sowing. Two different irrigation treatments were given. For *rainfed* conditions, irrigation was given only once at the time of sowing, whereas for restricted irrigation conditions, one additional irrigation was given 42 days after sowing. Data were collected on various phenological and physiological characteristics related with drought tolerance. Yield (YLD) and biomass (BIOM) were recorded in g/plot and harvest index (HI) in percentage. Primary yield contributing traits like productive tillers per meter (PT/M), grain number per spike (GR/S) were counted, plant height (HT) was measured in cm, grain weight per spike (GW/S), and 1000 kernel weight (TKW) were recorded in grams. Phenological traits like days to heading (DTH), days to maturity (DTM), and grain filling duration (GFD) were recorded in days. Physiological parameters such as canopy temperature (CT), Chlorophyll content (SPAD) and Normalized difference vegetation index (NDVI) were also recorded at anthesis. CT indirectly measures the evaporative cooling of the plants under drought, which influence the plants' photosynthetic efficiency. Lower values indicated cooler canopies. Canopy temperature was recorded thrice by using a handheld infrared thermometer. Chlorophyll content was recorded on three flag leaves by Minolta SPAD-502 chlorophyll meter and expressed in terms of SPAD units. NDVI was measured using a Field Scout

NDVI CM-1000 meter. An average of three readings was used for statistical analysis. The higher the value is, the more photosynthetic efficiency. Eight selection indices for stress tolerance, including harmonic mean (HM), mean productivity (MP), geometric mean productivity (GMP), tolerance (TOL), yield index (YI), yield stability index (YSI), stress tolerance index (STI), stress susceptibility index (SSI) were studied. These stress indices were calculated using the following formulas

Harmonic mean (HM)²⁶

$$HM = \frac{2(Y_p \times Y_s)}{(Y_p + Y_s)}$$

Mean Productivity (MP)^{18,27}

$$MP = \frac{Y_p + Y_s}{2}$$

Geometric mean productivity (GMP)¹⁷

$$GMP = \sqrt{Y_p \times Y_s}$$

Tolerance (TOL)^{18,27}

$$TOL = Y_p - Y_s$$

Yield index (YI)^{28,19}

$$YI = \frac{Y_s}{Y_p}$$

Yield stability index (YSI)²⁰

$$YSI = Y_s / Y_p$$

Stress tolerance index (STI)¹⁷

$$STI = \frac{Y_s}{Y_p^2}$$

Stress susceptibility index (SSI)¹⁴

$$SSI = \frac{1 - (\bar{Y}_s / \bar{Y}_p)}{1 - (\bar{Y}_s / \bar{Y}_p)}$$

where Y_s and Y_p are the mean yield of genotypes under *rainfed* and restricted irrigation conditions respectively and \bar{Y}_s and \bar{Y}_p are the mean yield of all genotypes under stress and non-stress conditions. The genotypes with high values of HM, MP, GMP, YI, YSI and STI are more desirable. The genotypes with low values of TOL are more stable in two different conditions. The genotypes with $SSI < 1$ are likely to be more resistant to drought stress conditions.

Table 1: Mean squares for morphological, physiological and agronomic traits under rainfed and restricted irrigation condition

Source	DF	Mean Square													
		DTH	DTM	GFD	CT	NDVI	SPAD	HT	BIOM	YLD	HI	TKW	GR/S	GW/S	PT/M
Rainfed condition															
Rep	2	0.43	1.28	3.13	1.7794	0.007	0.1483	18.8	278780.	33283.	7.42	12.43	30.474	0.40	446.4
		75	65	02		9***	177*		0677***	9844***	19	75***		07***	531*
Block	7	14.25	12.44	3.556	5.157	0.02	11.36	131.16	395500.	66139.05	38.76	39.94	65.97	0.10	661.9
		89***	05***	5***	5***	04***	96*	37***	9754***	88***	25***	57***	54***	38**	018***
Genotype	63	34.03	47.25	4.2	1.087	0.00	22.09	175.75	55839.8	12374.6	38.88	55.11	78.92	0.13	203.6
		05***	21***	3***	9***	23***	13***	63***	257***	015***	13***	98***	26***	4***	216***
Error	119	0.54	0.76	0.76	0.449	0.00	4.457	5.88	21707.	3660.	4.96	0.91	16.0	0.02	101.0
		79	79	75		07		28	3835	7074	02	63	754	86	541
Restricted irrigation condition															
Rep	2	12.796	4.70	3.812	5.606	0.00	2.67	9.43	37003.	20565.	97.64	11.78	51.5	0.04	246.1
		9***	31**	5**	9***	91***	69	75	5.833	66.15***	32***	65**	99	55	302**
Block	7	11.15	7.415	4.439	2.51	0.00	9.56	174.3	168040.5	25694.1	39.82	21.25	49.7	0.09	59.3
		48***	9***	7***	6***	25***	83	207***	804***	429***	67***	52***	232	94*	69
Genotype	63	51.4	39.467	7.080	0.729	0.00	17.32	215.36	59240.7	12498.4	35.31	45.050	69.23	0.136	229.5
		9***	6***	9***	7***	14***	93***	33***	419***	084***	96***	1***	18***	6***	075***
Error	119	0.49	0.9	0.65	0.17	0.0	5.66	5.14	14770.	2679.	3.64	1.628	24.7	0.04	47.67
		12	048	67	57	003	02	45	2858	9.989	64		951	48	31

*p<0.05, **p<0.01, ***p<0.001

DTH - days to heading, DTM – days to maturity, GFD - grain filling duration, CT- canopy temperature, NDVI - normalized difference vegetation index, SPAD - chlorophyll content, HT- plant height, BIOM- biomass , YLD – grain yield, HI – harvest index, TKW- thousand kernel weight, GR/S –grains per spike, GW/S – grain weight per spike, PT/M – productive tillers per meter.

Table 2: Mean comparison of yield and other traits under rainfed and restricted irrigation conditions for selected genotypes and checks

Genotype	DTH	DTM	GFD	CT	NDVI	SPAD	HT	BIOM	YLD	HI	TKW	GR/S	GW/S	PT/M		
<i>Rainfed</i>	DH 807	54	87	33	29.0	0.78	54.4	67	1225.3	477.7	39.0	31.7	48	1.60	76	
	DH 989	60	91	31	29.6	0.82	56.3	62	1268.0	469.0	37.0	26.7	49	1.33	82	
	DH 554	62	96	34	29.3	0.83	53.9	75	1299.3	440.7	33.4	26.3	45	1.23	73	
	DH 431	52	85	33	29.0	0.77	57.6	78	1189.3	440.0	36.8	39.3	41	1.80	73	
	DH 601	63	94	32	29.6	0.85	53.0	73	1228.0	412.0	33.6	25.0	42	1.30	77	
	DH 435	53	86	32	30.4	0.82	58.8	72	1094.7	405.0	37.0	36.7	40	1.60	58	
	DH 652	63	95	32	29.2	0.81	53.0	69	1254.7	405.0	32.4	24.0	50	1.50	62	
	DH 560	63	94	32	29.4	0.86	51.5	71	1196.7	403.0	33.7	26.7	44	1.30	81	
	DH 430	53	85	32	29.9	0.78	58.5	71	1069.3	401.7	37.6	38.3	40	1.77	63	
	DH 791	54	87	33	29.8	0.79	58.9	65	1105.3	397.3	35.7	31.7	48	1.53	59	
	NI 5439*	59	94	35	28.4	0.86	52.4	86	1327.3	499.3	37.6	29.0	43	1.40	85	
	NIAW 1415*	60	93	33	29.0	0.85	47.2	79	1439.3	489.0	33.9	28.0	46	1.40	98	
	DPW 621-50*	61	96	35	28.0	0.81	53.0	71	1362.0	484.3	35.6	29.0	48	1.40	59	
	MACS 6222*	57	91	34	29.1	0.85	54.5	72	1287.3	478.0	37.0	32.7	52	1.80	66	
	HD 2781*	57	91	34	29.8	0.79	53.9	70	1179.3	433.3	36.5	38.7	36	1.53	77	
	HI 1500*	58	91	33	29.4	0.80	50.6	99	1250.7	402.3	31.5	32.0	39	1.40	59	
	C 306*	63	99	37	29.1	0.88	52.5	91	1448.0	396.7	27.1	31.0	44	1.53	73	
	PBW 175*	61	95	34	28.3	0.84	53.9	72	1200.0	361.7	29.9	33.0	27	0.83	79	
	HI 1531*	62	98	36	29.3	0.83	52.4	76	1120.0	325.0	29.0	25.0	44	1.30	73	
	K 9644*	61	94	33	29.5	0.82	55.1	80	982.0	323.7	32.9	29.7	52	1.73	60	
	HW 2004*	64	99	35	29.5	0.83	50.7	82	1192.0	302.3	25.2	30.0	37	1.10	65	
	LSD @ 5%	1.196	1.317	1.228	1.028	0.049	2.844	3.957	263.667	108.47	3.463	1.391	5.798	0.245	14.95	
	Restricted Irrigation	DH 807	54	89	35	28.8	0.86	55.2	75	1596.7	618.7	38.8	28.3	53	1.73	80
		DH 430	51	87	36	28.4	0.87	57.6	88	1587.3	585.3	36.9	36.0	43	1.63	75
DH 435		53	90	36	28.5	0.87	55.7	87	1622.7	584.0	35.9	33.3	47	1.77	91	
DH 417		53	86	33	28.3	0.86	57.8	97	1535.3	546.0	35.6	38.7	43	1.80	67	
DH 431		52	88	36	28.5	0.83	54.2	85	1503.3	540.0	35.7	34.0	45	1.90	83	
DH 432		53	90	36	28.6	0.86	57.7	87	1462.0	500.3	34.5	35.3	45	1.67	94	
DH 791		55	90	35	28.7	0.86	54.3	75	1360.7	498.7	36.5	29.3	49	1.57	87	
DH 931		65	98	33	28.4	0.91	46.5	78	1567.3	487.3	30.8	26.3	45	1.20	93	
DH 601	65	98	33	27.7	0.90	53.8	85	1722.7	483.7	28.0	27.3	52	1.40	85		

DH 621	64	98	34	27.6	0.89	54.1	84	1635.3	476.3	29.1	23.7	53	1.53	103
MACS 6222*	60	96	36	28.0	0.87	56.0	85	1630.0	575.3	35.3	29.7	59	1.93	93
NIAW 1415*	61	96	35	27.5	0.90	52.7	91	1756.7	539.3	30.6	25.0	50	1.67	96
DPW 621-50*	62	99	37	27.6	0.86	49.4	76	1654.0	530.3	32.0	27.3	48	1.57	80
NI 5439*	60	98	38	27.7	0.89	52.3	97	1528.0	514.0	33.6	27.7	40	1.70	95
HI 1500*	59	97	38	27.4	0.89	54.3	109	1673.3	497.7	29.8	33.7	44	1.47	89
PBW 175*	62	98	36	27.9	0.89	51.0	88	1657.3	462.0	27.9	28.7	31	1.30	94
HD 2781*	58	95	37	27.8	0.89	56.1	91	1474.0	449.0	30.4	30.3	37	1.27	87
C 306*	65	102	37	27.6	0.93	54.0	111	1806.0	432.3	24.0	29.0	38	1.43	106
K 9644*	61	97	35	28.0	0.91	55.8	97	1595.3	431.0	27.2	26.3	55	1.60	76
HW 2004*	66	102	36	27.3	0.92	52.9	105	1578.7	384.0	24.3	28.3	51	1.67	100
HI 1531*	64	101	37	27.7	0.91	51.7	90	1721.3	364.3	21.1	21.7	46	1.20	96
LSD @ 5%	1.155	1.335	1.212	0.66	0.038	3.184	3.264	184.906	78.661	2.979	1.743	6.763	0.287	9.132

*checks; DTH - days to heading, DTM - days to maturity, GFD - grain filling duration, CT- canopy temperature, NDVI - normalized difference vegetation index, SPAD - chlorophyll content, HT- plant height, BIOM- biomass, YLD – grain yield, HI – harvest Index, TKW- thousand kernel weight, GR/S –grains per spike, GW/S – grain weight per spike, PT/M - productive tillers per meter

Table 3: Tolerance Indices of wheat genotypes under rainfed and restricted irrigation conditions. The numbers in the parenthesis are the genotype ranks for each index.

Sr.No.	Genotype	Yp	Ys	HM	MP	TOL	STI	SSI	GMP	YI	YSI
1	DH 5	341.00(62)	261.00(57)	295.68(59)	301.00(62)	80.00(30)	0.572(60)	1.183(43)	298.33(60)	0.746(57)	0.765(43)
2	DH 8	368.33(54)	300.33(50)	330.87(52)	334.33(51)	68.00(24)	0.711(52)	0.931(30)	332.60(52)	0.858(50)	0.815(30)
3	DH 15	417.66(35)	302.66(48)	350.98(42)	360.16(42)	115.00(49)	0.812(42)	1.388(49)	355.54(42)	0.865(48)	0.724(49)
4	DH 25	384.66(46)	322.00(40)	350.55(43)	353.33(43)	62.66(22)	0.796(43)	0.821(23)	351.94(43)	0.920(40)	0.837(23)
5	DH 31	395.66(42)	339.00(34)	365.14(40)	367.33(40)	56.66(20)	0.862(40)	0.722(20)	366.23(40)	0.969(34)	0.856(20)
6	DH 62	365.00(56)	240.00(61)	289.58(61)	302.50(61)	125.00(51)	0.563(61)	1.727(61)	295.97(61)	0.686(61)	0.657(61)
7	DH 66	393.66(44)	219.00(64)	281.43(63)	306.33(60)	174.66(62)	0.554(63)	2.237(64)	293.62(63)	0.626(64)	0.556(64)
8	DH 129	380.33(49)	319.33(41)	347.17(45)	349.83(46)	61.00(21)	0.780(46)	0.808(22)	348.50(46)	0.913(41)	0.839(22)
9	DH 134	378.00(50)	255.00(58)	304.54(58)	316.50(58)	123.00(50)	0.619(58)	1.641(57)	310.46(58)	0.729(58)	0.674(57)
10	DH 166	370.33(52)	293.00(53)	327.15(53)	331.66(53)	77.33(28)	0.697(53)	1.053(36)	329.40(53)	0.837(53)	0.791(36)
11	DH 173	325.00(64)	237.00(62)	274.11(64)	281.00(64)	88.00(34)	0.495(64)	1.365(48)	277.53(64)	0.677(62)	0.729(48)
12	DH 179	355.00(61)	244.00(60)	289.21(62)	299.50(63)	111.00(47)	0.556(62)	1.576(52)	294.31(62)	0.697(60)	0.687(53)

13	DH 189	372.66(51)	287.33(55)	324.48(55)	330.00(54)	85.33(33)	0.688(54)	1.154(42)	327.23(54)	0.821(55)	0.771(42)
14	DH 212	369.00(53)	279.33(56)	317.96(56)	324.16(56)	89.66(35)	0.662(56)	1.225(45)	321.05(56)	0.798(56)	0.757(45)
15	DH 227	397.00(41)	235.33(63)	295.50(60)	316.16(59)	161.66(58)	0.600(59)	2.053(63)	305.65(59)	0.672(63)	0.592(63)
16	DH 232	365.33(55)	336.00(36)	350.05(44)	350.66(45)	29.33(9)	0.789(44)	0.404(9)	350.35(44)	0.960(36)	0.919(9)
17	DH 256	409.66(38)	387.33(18)	398.18(28)	398.50(29)	22.33(7)	1.020(28)	0.274(6)	398.34(28)	1.107(18)	0.945(6)
18	DH 285	476.33(15)	315.00(46)	379.22(33)	395.66(30)	161.33(57)	0.964(31)	1.708(60)	387.35(31)	0.900(46)	0.661(60)
19	DH 370	361.33(58)	319.33(42)	339.03(48)	340.33(49)	42.00(14)	0.741(49)	0.586(17)	339.68(49)	0.913(42)	0.883(17)
20	DH 394	431.66(32)	376.33(20)	402.10(26)	404.00(27)	55.33(19)	1.044(26)	0.646(18)	403.05(26)	1.076(20)	0.871(18)
21	DH 412	397.66(40)	356.33(28)	375.86(36)	377.00(38)	41.33(13)	0.910(37)	0.524(14)	376.43(37)	1.018(28)	0.896(14)
22	DH 414	440.00(30)	339.33(33)	383.16(32)	389.66(32)	100.66(43)	0.959(32)	1.153(41)	386.40(32)	0.970(33)	0.771(41)
23	DH 415	455.66(23)	360.00(26)	402.22(25)	407.83(25)	95.66(38)	1.054(25)	1.059(37)	405.01(25)	1.029(26)	0.790(37)
24	DH 417	546.00(5)	375.33(23)	444.85(13)	460.66(10)	170.66(61)	1.317(11)	1.576(53)	452.69(11)	1.073(23)	0.687(52)
25	DH 430	585.33(2)	401.66(15)	476.41(8)	493.50(7)	183.66(64)	1.511(8)	1.582(55)	484.88(8)	1.148(15)	0.686(54)
26	DH 431	540.00(6)	440.00(8)	484.89(6)	490.00(8)	100.00(41)	1.527(6)	0.933(31)	487.44(6)	1.258(8)	0.814(31)
27	DH 432	500.33(10)	338.66(35)	403.92(23)	419.50(18)	161.66(59)	1.089(21)	1.629(56)	411.63(21)	0.968(35)	0.676(56)
28	DH 435	584.00(3)	405.00(11)	478.30(7)	494.50(6)	179.00(63)	1.520(7)	1.545(51)	486.33(7)	1.158(11)	0.693(51)
29	DH 483	387.66(45)	350.00(31)	367.87(39)	368.83(39)	37.66(11)	0.872(39)	0.490(13)	368.35(39)	1.000(31)	0.902(13)
30	DH 533	450.33(26)	358.66(27)	399.30(27)	404.50(26)	91.66(36)	1.038(27)	1.026(34)	401.89(27)	1.025(27)	0.796(34)
31	DH 554	467.00(19)	440.66(7)	453.45(10)	453.83(11)	26.33(8)	1.322(10)	0.284(8)	453.64(10)	1.260(7)	0.943(8)
32	DH 560	415.66(37)	403.00(13)	409.23(21)	409.33(24)	12.66(2)	1.076(22)	0.153(3)	409.28(22)	1.152(13)	0.969(3)
33	DH 590	453.66(24)	376.00(21)	411.19(20)	414.83(20)	77.66(29)	1.096(20)	0.863(26)	413.01(20)	1.075(21)	0.828(26)
34	DH 601	483.66(14)	412.00(10)	444.96(11)	447.83(14)	71.66(25)	1.281(13)	0.747(21)	446.39(13)	1.178(10)	0.851(21)
35	DH 607	426.66(34)	354.33(30)	387.15(29)	390.50(31)	72.33(26)	0.971(30)	0.855(25)	388.82(30)	1.013(30)	0.830(25)
36	DH 617	467.66(18)	355.33(29)	403.83(24)	411.50(23)	112.33(48)	1.068(24)	1.211(44)	407.64(24)	1.016(29)	0.759(44)
37	DH 621	476.33(16)	377.66(19)	421.30(17)	427.00(17)	98.66(40)	1.156(17)	1.044(35)	424.14(17)	1.079(19)	0.792(35)
38	DH 624	457.33(22)	375.66(22)	412.49(19)	416.50(19)	81.66(31)	1.104(18)	0.900(28)	414.49(18)	1.074(22)	0.821(28)
39	DH 652	452.00(25)	405.00(12)	427.21(16)	428.50(16)	47.00(16)	1.176(16)	0.524(15)	427.85(16)	1.158(12)	0.896(15)
40	DH 791	498.66(11)	397.33(16)	442.26(14)	448.00(13)	101.33(44)	1.273(14)	1.024(33)	445.12(14)	1.136(16)	0.796(33)
41	DH 807	618.66(1)	477.66(5)	539.09(1)	548.16(1)	141.00(54)	1.899(1)	1.149(40)	543.61(1)	1.365(5)	0.772(40)
42	DH 850	395.33(43)	373.66(24)	384.19(31)	384.50(34)	21.66(6)	0.949(33)	0.276(7)	384.34(33)	1.068(24)	0.945(7)
43	DH 875	382.33(48)	254.66(59)	305.70(57)	318.50(57)	127.66(52)	0.625(57)	1.684(58)	312.03(57)	0.728(59)	0.666(58)
44	DH 896	450.00(27)	317.33(44)	372.19(37)	383.66(35)	132.66(53)	0.918(35)	1.486(50)	377.88(35)	0.907(44)	0.705(50)
45	DH 931	487.33(13)	319.00(43)	385.59(30)	403.16(28)	168.33(60)	0.999(29)	1.742(62)	394.28(29)	0.912(43)	0.654(62)
46	DH 936	462.00(20)	317.00(45)	376.00(34)	389.50(33)	145.00(55)	0.941(34)	1.582(54)	382.69(34)	0.906(45)	0.686(55)
47	DH 937	360.66(60)	295.66(52)	324.94(54)	328.16(55)	65.00(23)	0.685(55)	0.908(29)	326.55(55)	0.845(52)	0.819(29)

48	DH 940	405.66(39)	299.66(51)	344.70(46)	352.66(44)	106.00(45)	0.781(45)	1.317(47)	348.66(45)	0.856(51)	0.738(47)
49	DH 942	441.33(29)	292.00(54)	351.46(41)	366.66(41)	149.33(56)	0.828(41)	1.706(59)	358.98(41)	0.834(54)	0.661(59)
50	DH 989	474.33(17)	469.00(6)	471.65(9)	471.66(9)	5.33(1)	1.430(9)	0.056(1)	471.65(9)	1.341(6)	0.988(1)
51	DH 990	416.33(36)	342.66(32)	375.92(35)	379.50(36)	73.66(27)	0.917(36)	0.892(27)	377.70(36)	0.979(32)	0.823(27)
52	DH 993	361.33(59)	313.33(47)	335.62(50)	337.33(50)	48.00(17)	0.727(50)	0.669(19)	336.47(50)	0.895(47)	0.867(19)
53	DH 995	340.66(63)	325.33(37)	332.82(51)	333.00(52)	15.33(4)	0.712(51)	0.227(5)	332.91(51)	0.930(37)	0.954(5)
54	DPW 6	530.33(8)	484.33(3)	506.29(5)	507.33(4)	46.00(15)	1.651(4)	0.437(11)	506.81(4)	1.384(3)	0.913(11)
	21-50*										
55	HD 2781*	449.00(28)	433.33(9)	441.02(15)	441.16(15)	15.66(5)	1.250(15)	0.175(4)	441.09(15)	1.239(9)	0.965(4)
56	HI 1500*	497.66(12)	402.33(14)	444.95(12)	450.00(12)	95.33(37)	1.287(12)	0.966(32)	447.46(12)	1.150(14)	0.808(32)
57	HI 1531*	364.33(57)	325.00(38)	343.54(47)	344.66(47)	39.33(12)	0.761(47)	0.544(16)	344.10(47)	0.929(38)	0.892(16)
58	HW 20	384.00(47)	302.33(49)	338.30(49)	343.16(48)	81.66(32)	0.746(48)	1.072(38)	340.72(48)	0.864(49)	0.787(38)
	04*										
59	C 306*	432.33(31)	396.66(17)	413.73(18)	414.50(21)	35.66(10)	1.102(19)	0.416(10)	414.11(19)	1.134(17)	0.917(10)
60	K 9644*	431.00(33)	323.66(39)	369.70(38)	377.33(37)	107.33(46)	0.896(38)	1.255(46)	373.49(38)	0.925(39)	0.750(46)
61	MACS*	575.33(4)	478.00(4)	522.16(2)	526.66(2)	97.33(39)	1.767(2)	0.853(24)	524.41(2)	1.366(4)	0.830(24)
	6222										
62	NI 5439*	514.00(9)	499.33(1)	506.56(4)	506.66(5)	14.66(3)	1.649(5)	0.143(2)	506.61(5)	1.427(1)	0.971(2)
63	NIAW	539.33(7)	489.00(2)	512.93(3)	514.16(3)	50.33(18)	1.695(3)	0.470(12)	513.55(3)	1.398(2)	0.906(12)
	1415*										
64	PBW 175*	462.00(21)	361.66(25)	405.72(22)	411.83(22)	100.33(42)	1.074(23)	1.095(39)	408.76(23)	1.034(25)	0.782(39)

*checks; Yp – yield under restricted irrigation, Ys – yield under *rainfed* condition, HM – harmonic mean, MP- mean productivity, TOL- tolerance, STI – stress tolerance index, SSI – stress susceptibility index, GMP – geometric mean productivity, YI – yield index, YSI – yield stability index.

The data were subjected to analysis of variance followed by LSD tests for means comparisons using Agrobases software and Principal component analysis (PCA) and cluster analysis was computed using STAR software. Pearson's correlation coefficient was determined using R studio.

Results

The results of ANOVA for YLD, PT/M, GR/S, GW/S, TKW, DTH, DTM, GFD, BIOM, HT, CT, NDVI, SPAD and HI in RF and RI environments indicated that genotypic differences were the significant source of variance for these traits ($P < 0.001$) (Table 1). In case of yield under *rainfed* condition superior genotypes DH 807, DH 989, DH 554, DH 431 were at par with the highest yielding check NI 5439, NIAW 1415, DPW 621-50 and MACS 6222 and better performing than remaining seven check varieties at 0.05 level of significance ($P < 0.05$). Under restricted irrigation condition, the genotypes DH 807, DH 430 and DH 435 were best performing lines with higher yield levels than all check varieties at 0.05 level of significance ($P < 0.05$). (Table 2). The response of genotypes at each condition varied based on agronomic, yield contributing, and physiological traits. Biomass was highest in C 306 in both RF and RI, whereas lowest in DH 66 and DH 173 under RF and RI, respectively. HI values were highest in DH 807 for RF and RI conditions (Table 2). Tolerance indices were calculated based on the yield of genotypes as shown in Table 3. The lowest TOL was found in DH 989, followed by DH 560, NI 5439 and DH 995. Hence, these genotypes had a lower yield reduction under RF conditions. SSI was highest in DH 66 and lowest in DH 989. A greater TOL value was observed in DH 430, followed by DH 435, DH 66, DH 417, and DH 931, indicating that these genotypes had a more significant yield reduction under *rainfed* conditions and higher drought sensitivity. The highest HM, MP, STI and GMP indices were observed in DH 807. The highest YI was observed in NI 5439, NIAW 1415, DPW 621-50, MACS 6222, DH 807 and higher YSI in DH 989. DH 66 showed the lowest YI as well as YSI.

The correlation coefficients between yield under *rainfed* as well as restricted irrigation and various surrogate traits along with other quantitative drought tolerance indices were calculated to assess the most desirable drought tolerance criteria (Fig 1, Table 4). In the present study, a significant positive correlation was found between the surrogate traits

like GFD, SPAD, HT, BIOM, HI, TKW, GW/S and PT/M and Yp whereas DTH, DTM and CT were negatively correlated. Similarly, a significant positive correlation was seen between NDVI, HT, BIOM, HI, TKW, GR/S, GW/S, PT/M and Ys. DTH, DTM and CT showed negative correlation with Ys. A positive correlation was observed between height, biomass, grain yield, harvest index, thousand kernel weight, grain weight per spike and resistance indices like HM, MP, STI, GMP, YI and YSI under *rainfed* and restricted conditions which suggest that these parameters are suitable for selecting genotypes under drought conditions. NDVI and productive tillers per meter were positively correlated with HM, MP, STI, GMP, YI and YSI under RF conditions, and SPAD was positively correlated with these indices under restricted irrigation conditions. CT showed a positive association with TOL and SSI under *rainfed* conditions and a positive association with SSI under RI conditions. NDVI showed a negative correlation with TOL and SSI under RF conditions. These results specify that the genotypes with low CT and high NDVI usually have high-stress tolerance under *rainfed* conditions. The check variety NI 5439, which had a low TOL and SSI also had low CT and high NDVI under RF conditions. Height was significantly positively correlated with HM, MP, STI, GMP and YI under both conditions and YSI under RF conditions only. A perfect positive correlation was observed between YI and grain yield under *rainfed* conditions, which suggests this parameter is suitable for selecting genotypes under drought conditions. In this study, a general linear regression of grain yield under drought stress on YSI revealed a positive correlation between this criterion with a similar coefficient of determination ($R^2 = 0.381$) (Fig 2). The drought tolerant indices produced a genotype performance order. The selection based on a combination of indices may grant a more useful criterion for improving drought resistance of wheat, although correlation coefficients are useful to find the degree of overall linear association between any two attributes.^{7,29,30} Thus, a better approach than correlation analysis, such as a biplot, was used to identify superior genotypes for both stressed and non-stressed environments. Principal component analysis (PCA) reduces the dimensionality of large data sets that are otherwise difficult to interpret and helps increase interpretability.³¹ A PCA was performed to identify drought-tolerant/ susceptible genotypes.

Table 4: Simple correlation coefficient between yield, tolerance indices and traits

Traits	Yield	HM	MP	TOL	STI	SSI	GMP	YI	YSI
DTH (RF)	-0.28***	-0.46***	-0.49***	-0.35**	-0.49***	-0.18ns	-0.47***	-0.33**	0.18ns
DTH (RI)	-0.46***	-0.39**	-0.42***	-0.39**	-0.43***	-0.23ns	-0.40***	-0.26*	0.23ns
DTM (RF)	-0.24***	-0.46***	-0.49***	-0.33**	-0.49***	-0.15ns	-0.47***	-0.34**	0.15ns
DTM (RI)	-0.44***	-0.37**	-0.41***	-0.47***	-0.41***	-0.30*	-0.39***	-0.22ns	0.30*
GFD (RF)	0.01ns	-0.22ns	-0.23ns	-0.09ns	-0.21ns	0.00ns	-0.23ns	-0.18ns	0.00ns
GFD (RI)	0.19**	0.18ns	0.17ns	-0.06ns	0.19ns	-0.09ns	0.17ns	0.18ns	0.09ns
CT (RF)	-0.44***	-0.45***	-0.42***	0.27*	-0.43***	0.37**	-0.44***	-0.49***	-0.37**
CT (RI)	-0.26***	-0.37**	-0.36**	0.22ns	-0.34**	0.31*	-0.36**	-0.41***	-0.31*
NDVI (RF)	0.44***	0.32**	0.29*	-0.39**	0.28*	-0.47***	0.30*	0.40***	0.47***
NDVI (RI)	-0.03ns	-0.06ns	-0.08ns	-0.23ns	-0.10ns	-0.22ns	-0.07ns	0.01ns	0.22ns
SPAD (RF)	-0.02ns	0.14ns	0.16ns	0.25*	0.14ns	0.15ns	0.15ns	0.06ns	-0.15ns
SPAD (RI)	0.27***	0.46**	0.46**	0.04ns	0.45***	-0.14ns	0.46***	0.41***	0.14ns
HT (RF)	0.47***	0.49***	0.48***	-0.13ns	0.46***	-0.27*	0.48***	0.49***	0.27*
HT (RI)	0.26***	0.32*	0.32*	-0.01ns	0.30*	-0.11ns	0.32*	0.30*	0.11ns
BIOM(RF)	0.89***	0.73***	0.70***	-0.42***	0.70***	-0.62***	0.72***	0.80***	0.62***
BIOM(RI)	0.66***	0.61***	0.61***	0.05ns	0.58***	-0.16ns	0.61***	0.55***	0.16ns
YLD(RF)	1.000***	0.96***	0.94***	-0.36**	0.94***	-0.62***	0.95***	1.00***	0.62***
YLD(RI)	1.000***	0.90***	0.94***	0.35**	0.92***	0.04ns	0.92***	0.75***	-0.04ns
HI (RF)	0.73***	0.77***	0.77***	-0.10ns	0.78***	-0.32**	0.77***	0.75***	0.32**
HI (RI)	0.76***	0.64***	0.67***	0.38**	0.68***	0.17ns	0.65***	0.50***	-0.17ns
TKW (RF)	0.31***	0.48***	0.51***	0.36**	0.49***	0.16ns	0.50***	0.35**	-0.16ns
TKW (RI)	0.61***	0.55***	0.59***	0.37**	0.57***	0.15ns	0.57***	0.42***	-0.15ns
GR/S (RF)	0.16*	0.04ns	0.01ns	-0.23ns	0.04ns	-0.21ns	0.03ns	0.09ns	0.21ns
GR/S (RI)	0.01ns	-0.05ns	-0.06ns	-0.06ns	-0.02ns	-0.01ns	-0.05ns	-0.03ns	0.01ns
GW/S (RF)	0.40***	0.50***	0.52***	0.14ns	0.52***	-0.04ns	0.51***	0.43***	0.04ns
GW/S (RI)	0.41***	0.48***	0.50***	0.20ns	0.52***	0.06ns	0.49***	0.39**	-0.06ns
PT/M (RF)	0.46***	0.29*	0.25*	-0.47***	0.26*	0.51***	0.27*	0.40***	0.51***
PT/M (RI)	0.15*	0.05ns	0.04ns	-0.14ns	0.03ns	-0.12ns	0.05ns	0.09ns	0.12ns

*p<0.05, **p<0.01, ***p<0.001, ns-Non significant

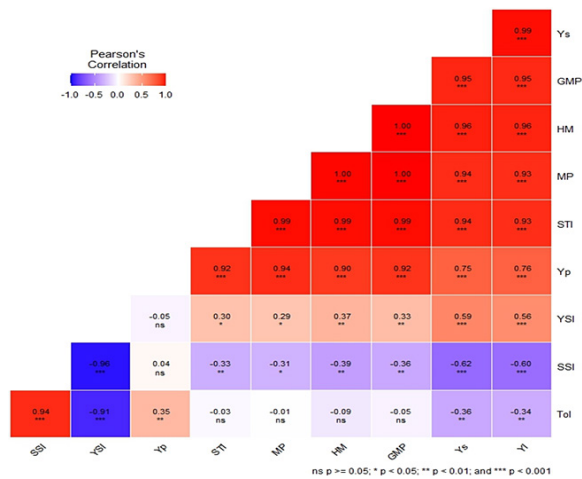


Fig 1: Correlation coefficients between Ys, Yp and resistance indices

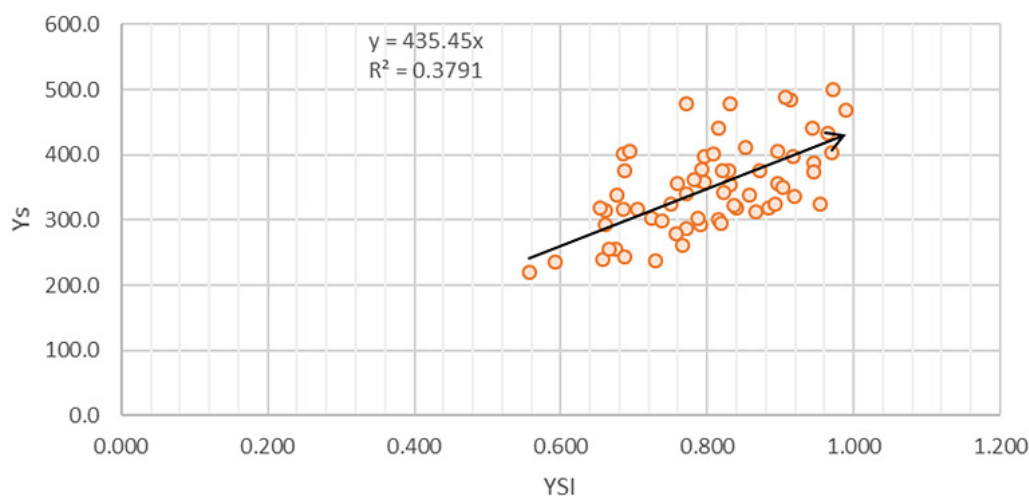


Fig 2: Relationship between drought stress grain yield and yield stability index (YSI)

The first two PCAs accounted for 99.7%, PC1-70.9 % and PC2-28.8 %, respectively (Fig 3). A close relation was observed between HM, GMP, STI and MP, indicating that they are approximately the same in the ranking of genotypes. A close angle between Ys and YI indicated that YI is identical to Ys in the ranking of genotypes (yield under stress conditions). The angle between GMP, HM, MP, STI, YI and YSI is an acute angle showing their close relationship with each other. Positive correlations were found between GMP, HM, STI and MP. These indices were also positively correlated with Ys (yield under stress) and Yp (yield under RI). TOL and SSI were strongly and positively correlated with each other, indicating that these indices can categorize the susceptible genotypes (i.e. DH 66, DH 227, DH931, DH 62, DH 942, DH 875, DH 134, DH 179). TOL and SSI showed a negative correlation with Ys, GMP, HM, MP, STI, YI and YSI based on which DH 989, DH 554, both tolerant genotypes could be distinguished. These genotypes performed well under *rainfed* and restricted irrigation, showing better performance than HD 2781 but below NI 5439 check varieties. A positive correlation was found between yield under *rainfed* and restricted irrigation, demonstrating that the genotypes under *rainfed* conditions could perform well under restricted irrigation. The check

varieties NI 5439, NIAW 1415, DPW 621-50 and genotype DH 989 were the most stable genotypes under unfavourable environments, while DH 807, DH 430, DH 435, MACS 6222 and DH 417 were highly adapted to restricted irrigation (Fig 3). Cluster analysis showed that the genotypes based on GMP, MP, HM, STI, YI, YSI, TOL and SSI tended to group into four groups with 6, 6, 25 and 27 genotypes, respectively (Fig 4). The cluster analysis illustrated that the first group consisting of DH 554, DH 989, HD 2781, NI 5439, DPW 621-50 and NIAW 1415 exhibited higher MP, HM, GMP, and STI. It was considered the most desirable cluster for both growth conditions with low SSI and TOL and high YSI, indicating their stability in performance. These results substantiate our previous findings derived from PCA. The second group (DH 417, DH 430, DH 435, DH 431, DH 807 and MACS 6222) showed genotypes with higher TOL, SSI and lower YSI values. Thus these genotypes were sensitive to drought and suitable for restricted irrigation conditions. The third group comprised mostly genotypes with high SSI and TOL values with low YSI and YI, indicating their poor performance under stress conditions. The fourth group was a moderate performer based on Yp, Ys, and the indices ranking.

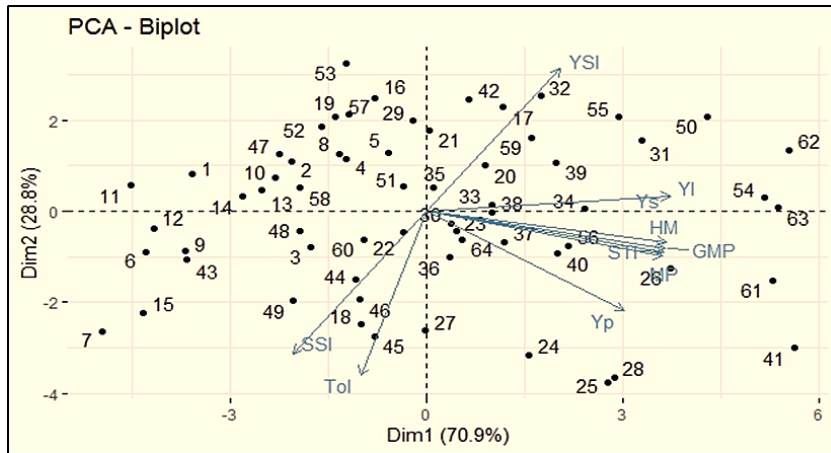


Fig. 3: Principal Component Analysis of drought tolerance indices

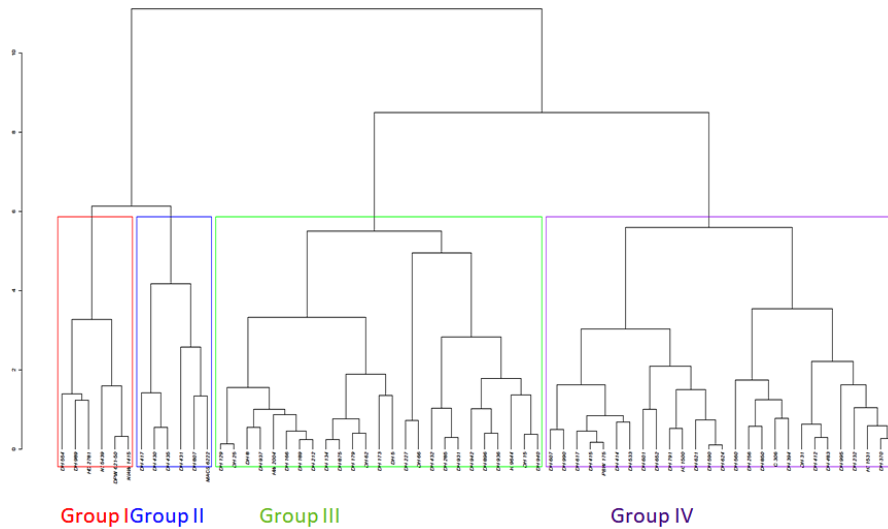


Fig. 4: Dendrogram using average linkage between groups showing classification of genotypes based on resistance indices

Discussion

Several agronomic and physiological traits influence grain yield response in wheat. Variation due to genotypes was significant for all traits in two conditions, viz., *rainfed* and restricted irrigation, which suggested that the magnitude of differences in genotypes was sufficient to provide some scope for selecting genotypes to improve drought tolerance. The mean comparison of traits observed in this study showed that DH 807 had the highest grain yield and harvest index under RI conditions which is similar to the inference drawn by Donald and Hamblin (1976)³² that genotypes with higher

HI exhibit higher yields. The present study showed a significant positive correlation of grain yield with agronomic traits like TKW, HI, BIOM, PT/M, GW/S and physiological traits like NDVI and a negative correlation with CT. Similar results were reported by Foulkes *et al.* (2007),³³ Lopes *et al.* (2012),³⁴ Beche *et al.* (2014),³⁵ Chen and Hao (2015),³⁶ Zhang *et al.* (2016),³⁷ and Gao *et al.* (2017).³⁸ The yield contributing traits like BIOM, PT/m, GR/S, and GW/S indicated that supportive irrigation during the reproductive phase enhances the yield and plays a key role in affecting the above traits. Moayedi *et al.* (2009)³⁹ and Garcia del Moral *et al.* (2005)⁴⁰

also noted similar results. GMP, MP, HM, YI and STI were significantly correlated with yield under both conditions (Table 4), indicating that these parameters are suitable to screen drought tolerant, high-yielding genotypes like DH 989. Similar results were reported by Fernandez (1992),¹⁷ Mohammadi *et al.* (2003),^{4,1} Golabadi *et al.* (2006),²⁹ Sio-Se Mardehet *et al.* (2006),¹⁰ Mohammadi *et al.* (2010),¹² and Nouri *et al.* (2011).⁷ Accordingly, these parameters were suitable for discriminating the best genotypes under both conditions. Mohammadi *et al.* (2010)¹² found that GMP, MP, and STI were positively correlated to yield under moderate stress. According to our study, the check genotype NI 5439 had high YI and YSI, which had a highly significant positive correlation with grain yield under *rainfed* conditions ($r = 1.0$ and 0.62 for grain yield and YI and YSI under *rainfed* conditions, respectively), hence these two parameters signify good tolerance to *rainfed* condition (Table 4). YSI was a valuable index for discriminating drought resistance from drought-susceptible genotypes.¹² A significant positive correlation was found between TOL and GY under restricted irrigation ($P < 0.01$), but this correlation was negative under *rainfed* conditions (Fig 1), indicating that the genotype with high GY under restricted irrigation had a high reduction in yield under the *rainfed* condition as revealed in DH 430. Similar results were reported by Nouri *et al.* (2011)⁷ and Mohammadi *et al.* (2010),¹² who showed that selection based on TOL would result in yield reduction under *rainfed* conditions. SSI was used by Clarke *et al.* (1992)¹⁵ to evaluate drought tolerance in wheat genotypes. He noted a year-to-year variation in SSI for genotypes and their ranking pattern. Bansal and Sinha (1991)⁴² used SSI and GY as stability parameters to identify drought-resistant genotypes in wheat. In this research, DH 989 and check NI 5439 had the lowest SSI, and therefore, these genotypes have low drought susceptibility and high yield stability in both conditions, whereas DH 66 followed by DH 227 with SSI higher than the unit, can be identified as having high susceptibility to drought. Similar results were reported by Golabadi *et al.* (2006),²⁹ Talebi *et al.* (2009)³⁰ and Nouri (2011),⁷ who showed that SSI could be a more useful index in discriminating better genotypes under *rainfed* conditions. In the present study, SSI and TOL showed a significant negative correlation with Ys ($r = -0.62$ and -0.36 , respectively). Larger SSI and TOL values represent relatively more sensitivity to stress. Hence smaller

SSI and TOL values are preferred. Selection based on these two criteria identifies genotypes with high yield potential under non-stressed conditions and low yield under stressed conditions (Fernandez, 1992).¹⁷ PCA was performed to evaluate the relationships between yield (Ys and Yp) and stress tolerance indices. The correlation coefficient among any two indices was approximated by the cosine of the angle between their vectors. Thus, $r = \cos 180^\circ = -1$, $\cos 0^\circ = 1$, and $\cos 90^\circ = 0$ (Yan and Rajcan, 2002).⁴³ The most prominent relations revealed by the biplots were (i) a strong negative association of TOL and SSI with YI, HM, GMP, MP and STI as indicated by the large obtuse angles between their vectors, (ii) a positive association of YSI with YI, HM, GMP, MP, STI and Yp with SSI and TOL as indicated by their acute angles, (iii) near to zero and non-significant correlation of Yp with SSI and YSI as indicated by the perpendicular vector. These results obtained from biplots confirmed the correlation analysis. The observation noted are in accordance with those reported by Fernandez (1992)¹⁷ in mungbean and Golabadi *et al.* (2006)²⁹ as well as Nouri *et al.* (2011)⁷ in durum wheat. In the present study, the check NI 5439 had the highest yield under RF condition, which exhibited high GFD, BIOM, HI and PT/M, high NDVI and low CT, and the highest values for YI and YSI and low values for SSI and TOL. DH 989 and DH 554 were selected as the most drought tolerant genotypes based on average ranks of stress indices. Finally, GMP and STI can be used to select drought-tolerant genotypes. The traits such as CT, NDVI, biomass, HI, productive tillers/meter, grain weight/spike and TGW are suitable for selecting the best genotypes under *rainfed* and restricted irrigation conditions as these parameters are strongly correlated with HM, MP, STI, GMP, YI, SSI and TOL. These parameters are useful for discriminating genotypes that have higher stability and lower susceptibility to stress conditions.

Conclusion

Yield and yield contributing traits under *rainfed* and restricted irrigation were independent. STI, GMP and MP were used to identify tolerant genotypes that produced high yields under both conditions. Based on YI and YSI, resistant genotypes could be discriminated which remain stable under different conditions and produce high grain yield under stress. The TOL value for genotypes was higher, yielding only under irrigated conditions. This study

indicated that some genotypes reduced grain yield under drought stress while others were tolerant to drought, which showed genetic variability for drought tolerance in this material which can be utilized by the breeders for the development of genotypes with better drought tolerance in assistance with the selection indices and physiological parameters. This could help to mitigate the disaster of climate change by developing a climate-smart and resilient variety.¹

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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