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PATTERN OF DIVERSITY FOR MORPHO-PHYSIOLOGICAL TRAITS UNDER TERMINAL HEAT STRESS CONDITIONS IN BREAD WHEAT

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ABSTRACT
One hundred twenty five recombinant inbred lines derived from the cross of WH 730 (thermotolerent) and WH 147 (thermosensetive), were evaluated to study the genetic diversity for morpho-physiological traits under terminal heat stress conditions. Eleven clusters were formed by grouping 125 RILs and their parents in such a way that genotypes within each cluster had smaller D^2 value than those in other clusters. Differences in proportion of contribution of each character to total diversity were observed and plant height ranked first by contributing 35.17% to divergence of genotypes, followed by membrane thermostability (27.18%), stomatal conductance (15.24%), days to heading (8.72%) and canopy temperature depression (3.85%). The principal component analysis showed that the first four principal components could account for 74.90 percent of the total variation and mainly associated with plant height, chlorophyll fluorescence, grain yield per plant, membrane thermostability, canopy temperature depression, days to heading, plant height and days to maturity.

KEY WORDS: Recombinant Inbred Lines, genetic diversity, heat stress, canopy temperature depression, membrane thermostability and chlorophyll fluorescence.

INTRODUCTION

Wheat, the most widely grown crop in the world in terms of total harvested area (Leff *et al*., 2004), is an essential component of the global food security by contributing about one-fifth of human caloric consumption (Shiferaw *et al*., 2013). High temperature stress has numerous effects on plants in terms of physiology, biochemistry and gene regulation pathways (Bita and Gerats, 2013). In wheat, a yield loss of 3–17% for each degree rise in temperature is estimated (Lobell *et al.,* 2008). High temperature can damage the inter-molecular interactions needed for proper growth, leading to catastrophic loss of crop productivity, thus remain as a serious serious challenge in sustaining high production. Acquired thermotolerance is a well-known adaptive phenomenon, refers to the ability of an organism to cope with excessively high temperatures. In view of global warming and changing scenario of the environmental conditions, it is imperative to direct breeding approaches toward developing wheat varieties adapted to warm temperatures. Existence of genetic variability in heat stress tolerance plays a crucial importance in relation to the development of more tolerant cultivars. Comprehensive and in-depth knowledge of divergence helps in framing a successful breeding programme. Estimation of degree of divergence between biological population and computation of relevant contribution of different components to the total divergence plays a vital role in planning breeding programme to develop superior cultivars. Different high temperature stress-related traits have received considerable attention, in particular cell membrane stability (Blum and Ebercon, 1981; Dhanda and Munjal, 2006), chlorophyll fluorescence (Moffatt *et al*., 1990 and Sayed, 2003), canopy temperature depression (Reynolds *et al*., 1998) and stomatal conductance (Munjal and Rana, 2003 and Bahar *et al*., 2009) provide a gain to screen wheat genotypes under heat stress conditions. However, yield and yield components in stress condition are still the most effective tools for stress evaluation (Ozkan *et al*., 1998).

MATERIALS & METHODS

Creation of heat stress environments

The experiment was carried out in Complete Randomized Block Design with three replications and conducted at CCS HAU, Hisar (29°10N′ lat., 75°46′E long., 215 m alt.). The plot size was of single row of 2.5 m in length with the spacing of 10 cm × 22.5 cm. In order to create heat stress at anthesis and during reproductive stages, the sowing of the heat stress experiment was delayed by about one and half month from normal period of sowing *i.e*. at last week of December.

Plant materials

The present investigation was carried out on 125 RILs of bread wheat derived from the cross WH 730 (a thermo-tolerant variety) and WH147 (a thermo-sensitive variety). Data were recorded as the average of five competitive plants selected randomly from each row.

Canopy temperature depression

A hand held infrared thermometer, (model AG-42, Tele temp crop, Fullerton CA) used for instantaneous measurement of canopy minus air temperature as canopy temperature depression.

Membrane thermostability

For membrane thermostability, method given by (Sullivan, 1972), modified later by Ibrahim and Quick (2001) was followed. Membrane thermostability was measured by the formula given below.

$$MTS = (1 - \frac{T_1}{T_2}) \times 100$$

T_1 = initial conductance value taken after incubation of leaf discs in a controlled temperature water bath at 49°C for 45 min followed by cooling.

T_2= final conductance value taken after autoclaved at 0.01 M Pa pressure for 10 min to release all the electrolytes from the leaf discs.

Chlorophyll fluorescence

The Chlorophyll fluorescence measurements, F_O (minimum fluorescence), Fm (maxmimum fluorescence) and Fv/Fm (maximum quantum yield of photosystem-II) were recorded from flag leaves using a portable handy chlorophyll fluorescence meter (model OS-30 p, Opti sciences, USA).

Stomatal conductance

Stomatal conductance (µmol m^{-2} sec^{-1}) was measured by using portable Infra Red Gas Analyser (IRGA): Li-Cor 6400, between 10.00 to 12.00 a.m.

Mahalonobis (1936) D^2 statistic analysis was used for assessing the genetic divergence among the RIL's involving phenological and yield component traits. D^2 values were clustered using Tocher's method as described by Rao (1952).

RESULTS & DISCUSSION

The results of ANOVA for dispersion indicates that mean sum of squares due to genotype were significant (Table 1). This revealed that there was a considerable amount of variability for all the characters under study among the RILs of bread wheat. Based on the results of diversity analysis, 125 RILs and their parents genotypes were grouped into eleven clusters by Non-hierarchical Euclidean Cluster Statistic in such way that the genotypes within a cluster had a small or low D^2 values than those of in between the clusters (Table 2).

TABLE 1: Analysis of variance for dispersion in recombinant inbred lines of bread wheat

Source of Variations	df	Sum of Squares	Mean Squares	F Ratio	Probability
Genotype	126	$2.4073E^{17}$	$1.9105E^{15}$	$9.999E^{03}$	0.00000 **
Error	251	$2.0635E^{-07}$	$8.2213E^{-10}$		
Total	377	$2.4073E^{17}$	$6.3853E^{14}$		

**: Significant at 1% level of significance

TABLE 2: Classification of recombinant inbred lines of bread wheat with their parents in different clusters

Cluster	No. of genotypes	Name of name of genotypes
I.	2	WH730 48
II.	15	4 110 112 109 64 102 70 105 65 91 98 103 80 107 75
III.	21	5 30 20 99 72 26 31 47 10 79 73 74 7 19 21 6 122 22 29 101 15
IV.	8	3 18 66 8 24 62 43 67
V.	30	9 14 83 90 53 125 44 52 51 34 46 45 39 111 77 40 42 54 55 60 95 120 94 41 127 63 89 96 126 38
VI.	9	11 123 23 113 114 117 118 119 WH147
VII.	5	33 115 116 69 104
VIII.	4	12 27 106 32
IX.	8	16 17 76 92 37 35 28 36
X.	20	25 124 97 85 87 57 78 108 93 121 61 86 49 56 59 82 68 81 71 100
XI.	5	58 84 88 13 50

TABLE 3: Average intra (diagonal) and inter (above diagonal) cluster D^2 values of recombinant inbred lines of bread wheat

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	5.873	7.015	6.915	8.029	7.581	7.37	8.131	8.199	8.574	7.672	9.287
II		4.692	5.062	5.954	5.654	6.302	6.953	7.346	7.176	5.866	7.312
III			4.206	5.529	5.421	6.435	7.204	6.951	6.636	5.542	7.329
IV				4.850	6.327	7.829	8.762	8.882	8.790	7.214	9.288
V					4.952	6.191	6.886	7.283	6.435	5.539	6.542
VI						5.273	6.582	7.289	7.564	6.487	7.488
VII							5.656	7.107	6.445	6.665	7.858
VIII								5.531	6.514	7.755	8.535
IX									4.624	5.791	6.188
X										4.696	5.532
XI											4.403

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TABLE 4: Cluster mean values of different characters in bread wheat recombinant inbred lines

Cluster	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
I.	7.289	3.556	214.444	3.389	2.159	63.028	13.927	19.833	25.170	101.060	0.293	89.500	122.000	5.133	55.321	0.759	100.778	341.33	0.340
II.	7.355	5.256	215.404	3.434	1.406	41.470	13.260	21.837	25.465	79.669	0.287	88.622	117.711	6.804	68.967	0.650	109.333	315.726	0.420
III.	7.078	5.701	246.22	2.984	1.253	43.048	13.047	21.386	24.965	78.595	0.288	87.349	116.714	6.464	71.648	0.681	115.437	371.326	0.257
IV.	3.608	4.347	107.694	3.365	0.845	26.072	12.300	21.014	17.968	71.321	0.217	88.375	117.667	5.529	66.006	0.660	117.588	360.289	0.313
V.	7.179	5.377	220.024	3.346	1.391	42.067	11.022	19.370	23.891	88.770	0.302	83.078	112.767	5.804	72.081	0.651	113.787	334.817	0.341
VI.	7.976	3.741	220.704	3.602	2.195	61.318	14.704	21.037	25.576	86.215	0.310	79.815	108.926	6.089	71.799	0.665	105.769	326.457	0.342
VII.	8.139	4.600	384.521	2.117	1.836	87.846	12.535	20.933	28.153	94.159	0.289	82.067	111.533	6.287	67.462	0.629	99.601	286.438	0.243
VIII.	8.485	3.667	359.917	2.357	2.446	106.583	11.631	19.417	22.913	73.324	0.370	87.000	116.583	7.458	73.886	0.656	116.211	356.547	0.335
IX.	10.586	6.958	514.418	2.106	1.550	74.170	10.208	18.375	33.399	93.049	0.317	84.042	113.458	6.942	73.234	0.653	114.455	339.913	0.354
X.	9.798	7.689	320.842	3.108	1.333	42.997	12.193	20.372	36.205	93.113	0.275	82.933	112.450	6.162	67.946	0.661	112.301	338.087	0.329
XI.	13.933	8.689	352.978	3.966	1.613	40.867	10.305	19.533	41.904	103.997	0.331	81.533	111.267	6.693	76.063	0.632	112.769	338.910	0.396

Where as,

1. Grain yield per plant, 2. No. of tillers per plant, 3. No. of grains per plant, 4. 100-grain weight, 5. Grain weight per spike, 6. No. of grains per spike, 7. Spike length, 8. No. of spikelets per spike, 9. Biomass, 10. Plant height, 11. Harvest index , 12. Days to heading, 13. Days to maturity, 14. Canopy temperature depression, 15. Membrane thermostability, 16. Ratio of variable fluorescence to maximal fluorescence- Fv/Fm , 17. Original fluorescence Fo , 18. Maximum Fluorescence - Fm, 19. Stomatal conductance

Cluster pattern revealed that, cluster V was the largest consisting of 30 genotypes. This way followed by cluster III (21 genotypes), cluster X (20 genotypes) , cluster II (15 genotypes), cluster VI (9 genotypes), cluster IV (8 genotypes) and cluster IX (8 genotypes), cluster VII (5 genotypes) and cluster XI (5 genotypes), cluster VIII (4 genotypes), cluster I (2 genotypes). The intra and inter cluster distances are given in Table 3. A maximum difference among the genotypes within the same cluster was shown by cluster I (5.873). This was followed by cluster VII (5.656), cluster VIII (5.531), cluster VI (5.73), cluster V (4.952), cluster IV (4.850), clusters X (4.696), cluster II (4.692), clusters IX (4.624), clusters XI (4.403). When diversity within clusters was studied, it showed a range of 5.062 to 9.288. Cluster IV and XI showed maximum inter cluster distance of 9.288, followed by that between clusters I and XI (9.287). The lowest inter cluster distance was noticed between clusters III and II (5.062), followed by that between clusters V and III (5.421).

It was evident that grain yield per plant was the highest in cluster-XI (13.933) and lowest in cluster-IV (3.6089) (Table 4). Canopy temperature depression exhibited highest mean value in cluster VII (7.458) and lowest mean value in cluster I (5.11). The mean value of membrane thermostability varies from 76.063 in cluster XI to 55.321 in cluster I. The cluster I (0.759) and cluster VII (0.629) showed the highest and lowest mean values for Fv/ Fm. For stomatal conductance cluster II (0.42) revealed the maximum mean value and cluster VII (0.243) minimum mean value. Overall cluster XI had the highest mean value for six characters. Therefore, cluster XI was considered most desirable for selecting genotypes. While the genotypes of the cluster VI have highest spike length, along with earliest in days to 50% flowering. The genotypes of cluster VI could be crossed with the genotypes of cluster XI for getting desirable transgressive segregants and high heterotic response. As for degree of contribution of each character to divergence (Table 5), plant height (ranked first, 2814 times out of 8001 total number of combinations) contributed 35.17% to divergence of genotypes. This was followed by membrane thermostability (27.18%), stomatal conductance (15.24%), days to heading (8.72%) and canopy temperature depression (3.85%). The minimum contribution toward total diversity was by grain weight per spike and Fo. Each of them contributed 0.02% of diversity. The above results were supported by Nimbalkar *et al*. (2002) and Chapla *et al.* (2011).

TABLE 5: Percent contribution of different traits towards total diversity

Sr no.	Source	Times Ranked 1st	Contribution %
1.	Grain yield per plant	9	0.11
2.	No. of tillers per plant	105	1.31
3.	No. of grains per plant	230	2.87
4.	100-grain weight	79	0.99
5.	Grain weight per spike	2	0.02
6.	No. of grains per spike	175	2.19
7.	Spike length	67	0.84
8.	No. of spikelets per spike	32	0.40
9.	Biomass	23	0.29
10.	Plant height	2814	35.17
11.	Harvest index	3	0.04
12.	Days to heading	698	8.72
13.	Days to maturity	48	0.60
14.	Canopy temperature depression	308	3.85
15.	Membrane thermostability	2175	27.18
16.	Fv/ Fm	4	0.05
17.	Fo	2	0.02
18.	Fm	8	0.10
19.	Stomatal conductance	1219	15.24

There are several reports about the existence of genetic diversity for heat tolerance in conventional wheat varieties (Gibson and Paulsen, 1999; Zhao *et al.,* 2008). Exploring new sources of genetic diversity must be continued. Expansion of genetic variability, in the wheat gene pool, aimed to improve heat tolerance, can be done by cross-breed wheat and genetic variability for heat tolerance amongst breeding lines can be identified. The transgressive segregants coming out of such crossing programme would be better adapted to heat stressed condition and careful selection is followed for trait specific and condition suited approach is implemented to make yield improvement in wheat for such conditions.

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