Efficiency of stress-adaptive traits, chlorophyll fluorescence and membrane thermo-stability in wheat under high temperature

Sheikh S, Behl RK and Dhanda SS

Department of Plant Breeding, CCSHAU, Hisar, INDIA

ABSTRACT

Heat stress affects at least 5 million ha of spring wheat in the developing world and over 7 million ha are grown in continual heat stress, with mean daily temperatures of greater than 17.5°c in the coolest month (Fischer and Byerlee, 1991). The demand for wheat is expected to grow by approximately 1.6 percent per year worldwide and by 2 percent per year in developing countries by the year 2020. This implies an urgent need for refinement of new and more efficient wheat breeding methodologies to identify new traits, particularly physiological traits, optimization of phasic development with respect to raising yield potential and to complement existing breeding techniques for warmer heat growing regions worldwide. The most important step is to recombine elite genotypes, introgression of genetically diverse DNA to incorporate *Vrn, Ppd,* and *Eps* genes, increase partitioning of photo-assimilates, translocation from stem to grain of soluble carbohydrates (stem reserve), ability to maintain green leaf area duration (stay green) and to improve radiation use efficiency throughout the grain filling period. Measurements of membrane thermo-stability and chlorophyll fluorescence in parents and F1's of six crosses at post- anthesis stage during 2006-07 were recorded. Acquired thermo-tolerance showed significant variation in late sown conditions for parents and Fl's. It was inferred that genotype PBW 435 and the cross PBW 343 x PBW 435 conferred less relative injury and greater thermo- tolerance possibly through maintaining cellular membrane integrity under high temperature stress. Data based on chlorophyll fluorescence revealed a reduction of mean value of all genotypes and their F1's for Fv/m, variable fluorescence in late sown conditions. The genotypes and Fl's showed high Fv/m values under both environments and displayed good tolerance to high temperature.

INTRODUCTION

Terminal heat stress can be a problem in up to 40 percent of irrigated wheat growing areas in the developing world. High temperature (> 30°C) at the time of grain filling is one of the major constraints in increasing productivity of wheat in tropical countries like India (Rane *et al.,* 2004). Fischar and Mourer in (1976) showed that a 1°C rise in temperature above ambient temperature during the period between the end of tillering and the beginning of grain filling reduced the grain yield by 4% under heat stress conditions. The demand for wheat is expected to grow by approximately 1.6 percent per year worldwide and by 2 percent per year in developing countries by the year 2020.This implies an urgent need for refinement of new and more efficient wheat breeding to recombine elite genotypes, introgression of genetically diverse DNA to incorporate *Vrn, Ppd,* and *Eps* genes, increase partitioning of photo-assimilates, translocation from stem to grain of soluble carbohydrates (stem reserve), ability to maintain green leaf area duration (stay green). The photosynthetic apparatus in plant is highly thermolabile and is damaged before visible symptoms of high temperature injury are manifested. High temperature affects thylakoid related reactions, changing the amount of absorbed light energy that is transduced from photo system II (PSII) to photo system I (PSI) and in turn, altering the pattern of chlorophyll fluorescence. Only a portion of light absorbed by a leaf at normal physiological temperature is used in the photosynthetic apparatus and the balance is dissipated as heat or reemitted as fluorescence.

MATERIALS AND METHODS

Nine genetically diverse homozygous genotypes of wheat (*Triticum aestivum* L. em. Thell) viz. PBW 343, WH 283, WH 542, PBW 435, UP 2565, UP 2425, EIGN1 and EIGN8 were selected to generate the experimental material. Experimental material comprised six parental and segregating generations (P_1, P_2, F_1, F_2, BC_1 and BS1) of each of the following six crosses,PBW 343 x WH 283,PBW 343 x WH 542,PBW 343 x PBW 435,UP 2565 x UP 2425,EIGN1 x Raj 3765 and EIGN8 x UP 2425. The experiment was conducted in the field at two dates of sowing viz.E_1 = Normal sown environment (1 Dec, 2006) and E_2 = Late sown environment (4 Jan-2007).

Chlorophyll fluorescence The data recorded for F_0, F_m and F_v/m for chlorophyll fluorescence was taken ~4 cm from the base of abaxial surface of primary tiller's flag leaf using a portable fluorometer (model SF-20, Richard Branker Research Ltd., Ottama) for parents and their F_1's in both normal and late sown environments at post anthesis stage. Measurements were taken on three randomly selected plants for parents and their F_1 hybrids in each three replications. In the present study, the objective was to determine differences in fluorescence parameters among parents and the F_1 generation in normal and late sown environments post anthesis.

Membrane thermostability, (Ibrahim and Quick, 2001) a random sample of flag leaves from three plants from each row for parents and their F_1's were collected at post anthesis stage (16^{th} April 07) in late sown environments. Each sample from the field was collected in a sealed plastic bag and immediately placed in an ice box. All the samples were thoroughly rinsed twice in deionized water. The midrib of the flag leaves were removed gently by hand and about 1 cm from the central flag leaf area was dissected into four equal parts to make a

homogeneous mixture. Two random samples from this homogeneous mixture were taken in glass test tubes with 10 ml deionized water for observation of the physiological trait. The test tube samples were tightly covered with aluminum foil and submerged in a water bath (maintained at 50ºC) to a depth equal to the height of water in the test tubes for 30 minutes. After the treatment period the test tubes were held overnight at room temperature. Conductance was measured with a conductivity meter. The test tube samples were then autoclaved, held at a pressure to 0.10 Mpa for 10 minutes to completely kill plant tissue and release all the electrolytes. The conductance was measured again after autoclaving. Membrane thermo stability was expressed in percentage units as the reciprocal of relative leakage:

MTS = (1-T_1/T_2) x 100

Where, **T1** = conductivity reading after heat treatment, **T2** = conductivity reading after autoclaving.

RESULT AND DISCUSSION

Chlorophyll fluorescence The mean values of Fv/m, variable fluorescence (Table 2), reduced in all the cases in the late sown environment compared to the normally sown environment. It was observed that the magnitude of Fv/m differences differed with a narrow range among all the six crosses in both the environments. The maximum F_v/m value was observed in WH 542 (0.751) followed by PBW 435 (0.737) and PBW 343 (0.736) and minimum in UP 2425 (0.693) in normal sown while in late sown, maximum levels were in UP 2565 (0.697) followed by UP 2425 (0 .691) and Raj 3765 (0.680) and minimum in EIGN8 (0.606). Among the F1's, the maximum value for chlorophyll fluorescence was observed in PBW 343 x WH 542 (0.739) followed by EIGN l x Raj 3765 (0.738) and minimum F_v/m values in EIGN 8 x UP 2425(0.694) in normal sown. On a percent reduction basis in F_v/m value of normal to late sown, a maximum of 15.5 per cent was found in EIGN 8 and minimum of 0.2 per cent in UP 2425. However, a maximum of 15.0 per cent was found in PBW 343 x PBW 435 and a lower 6.0 per cent in PBW 343 x WH 283 was found among the F_1's. The relative stability of Fv/m is indicated by small difference in percent reduction in UP 2425 (0.2) and UP 2565(2.0). UP 2565 followed by UP 2425 and Raj 3765 displayed good tolerance to high temperature conditions suggesting productivity (table-1) of these parents and F1's was directly related to photosynthetic apparatus during high temperature stress conditions. Similar findings were reported by Al-Khatib and Paulson, 1984; Moffatt *et al*., 1990a and b. The F1's performance in the case of UP 2565 x UP 2425 and EIGN 8 x UP 2425 could not be obtained either due to non- germination or poor survival of plants in the late sown,high temperature conditions. Hence, because of these two reasons and based on the findings, it is suggested that measurements should be recorded over different dates and a number of replications may also be needed for precise estimation and to determine the stability of the chlorofluorescence parameter.

Membrane Themostability (MTS)

The measurements for MTS (%) means of replicated data of parents and their F_1s, were recorded post anthesis in the late sown environment (table-2). The mean MTS values among the parents ranged from 44.8 percent in EIGN l to a maximum of 69.8 percent in WH 283. MTS ranged from 51.2 percent in PBW 343 x PBW 435 to 71.0 percent in PBW 343 x WH 542 among the F_1 crosses. Consistently, more than 55 percent membrane injury was recorded in all the parents except EIGN 1 and PBW 435, where the MTS values observed were 44.8 and 54.7 respectively. Only one intermediate type PBW 343 x PBW 435, exhibited low (51.2 percent) membrane injury. Based on the percent damage (less than 50%,50-60 % and more than 60%), PBW 343,WH 283,WH 542,UP2565 and EIGN 8 genotypes and crosses PBW 343 x WH 283 and PBW 343 x WH 542 were sensitive types and PBW 435, Raj 3765 and UP 2425 were moderate, tolerant types. Only one parental genotype EIGN 1(44.8%) was found to be a tolerant type. It was inferred that genotypes with less relative injury possessed greater thermo tolerance posibly through maintaining their cellular membrane integrity under high temperature conditions. Similar findings have also been reported by Shanahan *et al.,* 1990; Fokar *et al*.,1998; Ibrahim and Quick,2001; and Singh *et al*., 2005.

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Table 1: Mean performance of six crosses for grain yield (g)/plant under normal (E1) and Late sown (E2) environments

Characters	***Environme*nt**	**P1**	**P2**	**F1**
PBW 343 x WH 283	**E1**	**31.5**	**34.7**	**32.8**
	E2	**12.4**	**14.3**	**11.9**
PBW 343 x WH 542	**E1**	**34.2**	**28.4**	**30.6**
	E2	**15.6**	**14.8**	**8.35**
PBW 343 x PBW 435	**E1**	**35.8**	**34.9**	***31.9***
	E2	***10.6***	***15.3***	***12*.9**
UP 2565 x UP 2425	**E1**	**17.3**	**27.1**	**34.4**
E1 x Raj 3765	**E1**	**15.1**	**19.8**	**29.9**
	E2	**14.3**	**8.04**	**20.4**
E8 x UP 2425	**E1**	**16.6**	**18.3**	**16.3**
	E2	**16.1**	**15.6**	**19.0**

Table 2: Membrane Thermo stability (% relative injury) post-anthesis and means of Chlorophyll Fluorescence (F_0, Fm and F_v) in normal and late sown environments

Parents and F1's	MTS (%)	LEVEL	NORMAL SOWN			LATE SOWN			Percentage reduction over normal sown(%)
			Fo	Fm	Fv/m	Fo	Fm	Fv/m	
PBW 343	60.8	S	158	609	0.736	164	482	0.640	11.7
WH 283	69.8	S	153	598	0.735	160	509	0.679	7.7
WH 542	65.3	S	163	665	0.751	162	519	0.669	10.9
PBW 435	54.7	MT	166	648	0.737	158	473	0.652	11.5
UP 2565	63.8	S	155	543	0.711	169	571	0.697	2.0
UP 2425	57.8	MT	154	523	0.693	163	539	0.691	0.2
EIGN 1	44.8	T	152	561	0.728	162	490	0.665	8.6
Raj3765	55.1	MT	160	586	0.723	170	552	0.680	6.0
EIGN 8	66.7	S	151	549	0.717	160	442	0.606	15.5
PBW 343 x WH 283	67.7	S	148	548	0.715	157	495	0.672	6.0
PBW 343 x WH 542	71.0	S	160	620	0.739	162	490	0.664	10.1
PBW 343 x PBW 435	51.2	MT	144	539	0.727	155	423	0.618	15.0
UP2565 x UP2425	54.4	MT	152	505	0.694	-	-	-	-
EIGN 1 x Raj 3765	**60.2**		153	588	0.738	159	471	0.661	10.5
EIGN 8 x UP 2425			154	526	0.694	-	-	-	-
					0.722			0.661	

<50%RI:toleraent(T);50-60%RI:moderatetolerant(MT);and>60%RI:sensitive(S)sitive(S)