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Research Article

Alterations in cellular membrane stability due to heat stress in different genotypes of bread wheat

Radha Sharma[1], Richa Sharma[2], Jebi Sudan[1], Romesh K Salgotra[1] and Ravinder Singh[1]
***[1]School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha, Jammu,J&K, India-180 009
[2] Fermentation Technology Division, CSIR-Indian Institute of Integrative Medicine, Jammu
* Email : **rssandey@gmail.com**

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Abstract
Heat stress due to increase in temperature is an agricultural problem in many parts of the world. The objective of the present study was to examine changes in cell membrane stability when leaf samples of similar size of biological replicates from a single genotype of different varieties of wheat were exposed to varying temperatures (45°C and 100°C).The highest per cent of conductivity was observed in HDR77 as compared to remaining eleven varieties (HD 2864, HD 2967, PBW 373, HS 365, Raj 4037, JAUW 584, PBW 175, RSP 561, HW 2045, HD2385, HD2687) which predicts that highest susceptibility of this variety and least per cent of conductivity was seen in HD 2687 which predicts that it is highly resistant variety.

Key words
Bread wheat; heat stress; electrolyte leakage; tillering stage

Introduction

Bread wheat (*Triticum* sp.) is a self-pollinating, a hexaploid annual plant (AABBDD) with the chromosome number of 42 (2n=6x=42) belonging to the family *Poaceae* (grasses). Bread wheat (*Triticum aestivum* L.) is one of the major staple food crops of the world. Wheat is one of the essential winter cereal crops of India. Presently India ranks second in the world after China with the harvest of 93.9 million tons wheat from an area of 29.4 million hectare with the productivity of 2.86 tons per ha during the year 2012. The total wheat production for 2014-15 was approximately 86.53 million tonnes, which was lower than 2013-14 which was 95.85 million tonnes (According to Indian wheat database). Improper agronomic practices, poor management and unfavourable weather conditions such as high temperature, drought and salinity leads to a decrease in wheat production (Anonymous 2010). A transient increase in temperature usually 10–15°C above ambient, is considered as heat shock or heat stress. Most of the world crops are exposed to heat stress during certain stages of their life cycle (Stone 2001). Exposure to higher than optimal temperature reduces yield and decreases quality of cereals. As the world population is growing exponentially, there is a great need to increase productivity of the crop. These goals need significant breeding efforts to improve cereal yield and quality under high-temperatures.

Abiotic stress responses are very complex phenomenon as various stages of plant development can be affected by a particular stress and often several stresses simultaneously affect the plants (Chinnusamy *et al.,* 2004). This involves an array of physiological and biochemical changes in plants including wilting of leaf , decrease in leaf area, abscission of leaf, root growth stimulation, relative water content changes (RWC), electrolytic leakage (EL) and free radicals accumulation that disrupt cellular homeostasis by reacting with lipids, proteins, pigments and nucleic acids resulting in lipid peroxidation (LP), membrane damage, and the inactivation of enzymes, thus affecting viability of cells (Bartels and Sunkar 2005).

Three commonly used assays of heat tolerance in plants (Blum 1988) are related to the plasmalemma (cell membrane stability or CMS test), the photosynthetic membranes (chlorophyll fluorescence assay) and the mitochondrial membranes (cell viability assay based on 2,3,5-triphenyl-tetrazolium chloride (TTC) reduction). Leaf relative water contents (LRWC), leaf water potential, stomatal conductance and rate of transpiration are influenced by leaf and canopy temperature (Farooq *et al.*, 2009). Elevated temperatures may reduce the activities of antioxidant enzymes, as observed in maize. The integrity and functions of biological membranes are sensitive to high temperature, as heat stress alters the tertiary and quaternary structures of membrane proteins. The increased solute leakage, as an indication of decreased cell membrane thermostability (CMT), has long been used as an indirect measure of heat-stress tolerance in diverse plant species, including wheat (Blum *et al.*, 2001) and barley (Wahid *et al.*, 2007). Recent studies on bread wheat also identified some heat stress tolerant genotypes (Khajuria *et al.,* 2016).

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Electrolyte leakage is influenced by plant/tissue age, sampling organ, developmental stage, growing season, degree of hardening and plant species.

The cellular membrane dysfunction due to stresses is well expressed in increased permeability and leakage of ions out from the cell membrane which can be readily measured by the efflux of electrolytes. Thus, the estimation of membrane dysfunction under stresses by measuring cellular electrolyte leakage from affected leaf tissue into an aqueous medium is helpful for us for measuring CMS. The method was initially developed by the late C.Y. Sullivan (University of Nebraska) in the late 1960's for assessing sorghum and maize heat tolerance. Various studies have used electrolytes leakage test (Ismail *et al.,* 1997; Kumari *et al*., 2009) for verifying the membrane damage of the cell. The present study was being undertaken to achieve following objective *i.e*. screening of twelve genotypes of bread wheat for heat stress tolerance.

Materials and Methods

Plant Material

The research work was undertaken at the experimental farm, SKUAST-Jammu and molecular biology laboratory of School of Biotechnology, SKUAST-J, Main Campus, Jammu. The experiments were conducted during 2013-16. The experimental material for the study comprised of twelve genotypes (Table 1) of wheat (*Triticum aestivum* L) grown in the experimental field of. The genotypes were sown in a Randomised Block Design (RBD) with three replications for each genotype. Recommended package of practices were followed for raising a good crop.

Methodology

Electrolyte leakage measurement

For all the wheat genotypes, leaf discs or pieces of leaf tissue at tillering stage were sampled and placed in standard glass vials (50 ml capacity and of size 30 × 115 mm dimension) . The samples were harvested from all the three replications. The total area of leaf material per vial was about 15 to 25 cm^2. All the samples were washed 3 times with de-ionized water (distilled water). The samples were drained off retaining just enough water to keep them moist. Electrolyte measurement was measured following Fokar *et al.* 1998 with some modifications that included use of distilled water to each vial so that plant samples (of 0.5 gm of tillering stage) remains moist at all times. The leaf samples for each replicate was distributed into three vials, the first vial was kept at room temperature as a control without any heat treatment, the second one was subjected to heat stress at 45°C for 45 minutes and third was imparted to heat stress at 100°C for 15 minutes, respectively (Fig.1). The conductivity of the samples including control were measured after heat stress treatment using the conductivity meter (Eutech Instruments, Singapore). All the samples were cooled down to room temperature before conductivity measurements (Fig.1). The relative conductivity was measured using the following formula:

Relative Conductivity (% Conductivity) = (Conductivity at 45°C/Conductivity at 100°C) ×100

Membrane Stability Index (MSI) = 1- (Conductivity at 45°C/ Conductivity at 100°C) ×100

The analysis of variance (ANOVA) for electrolyte leakage was carried out using Microsoft Excel. ANOVA was used to determine the significant differences among the tested variables. Differences were considered to be significant at mean sum square value on the basis of F tabulated at 1% and 5% as compared to F calculated.

Results and Discussion

The electrolyte analysis of twelve wheat genotypes revealed higher leakage of cell electrolytes at 100ºC treatments compared to controls and treatment at 45ºC. Based on the conductivity meter reading, the highest conductivity was found HDR 77 and lowest for HD2687 and relevant MSI values were calculated for all wheat genotypes (Table 2). The analysis of variance analysis suggested that genotypes did differ in their response to heat stress. In ANOVA, MSS due to genotypes were significant at room temperature, 45ºC and 100ºC at 1% level of significance whereas for replication also were significant at room temperature and 100ºC (Table 3). The cell membrane stability analysis on the basis of electrical conductivity led to identification of HDR77 and HD2687 as the most susceptible and resistant varieties respectively in the present study. The electrolyte leakage (EL), as indicator on cellular membranes damage of *Triticum* seeds increased under heat stress (Al-Jebory, 2013). The studies have indicated a change in EC when experimental material was subjected to higher temperature even for a brief period. EC was (26.7, 28.5, 28.6, 30.4 and 33.9) µs/cm for seeds which treated with high temperature at (2, 5, 10, 15 and 20) seconds respectively as compared to control (17.9) µs/cm (Al-Jebory, 2013). This study was showing similar results with the results of present study. Electrical conductivity has been used as an index of membrane stability to identify heat-tolerant genotypes in wheat (Blum and Ebercon, 1981) and to screen of heat-tolerant genotypes in different crops (Blum, 1988). When tissues are subjected to high temperature, electrical conductivity increases due to damage to the cell membrane and consequent solute leakage. These previous studies (Kumar *et al.,* 2012) were showing similar results with the results of present study (Fig. 2, 3 and 4).

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Heat stress leads to severe changes in the cell membrane stability and ultimately influences the sensors present in the membrane of the cell. Cell membrane stability has been used to screen different wheat genotypes for heat tolerance (Blum and Ebercon, 1981; Saadalla *et al.,* 1990.). In this investigation we found that when leaf samples of tillering stage were kept at room temperature as control, their electrical conductivity was less as compared to electrical conductivity of leaf samples given stress at 45°C which were having less conductivity as compared to leaf samples given stress at 100°C. This reveals that high temperature leads to more membrane dysfunction and hence more efflux of electrolytes into the aqueous medium which results in more electrical conductivity values. Membrane damage through reactive oxygen species induced lipid peroxidation leads to the loss of cellular integrity, inactivation of membrane enzymes and to little extent even of cytoplasmic proteins. All these activities adversely affect the functioning of the plant cell. These changes during initial stages such as an increase in ROS generation, provides a signal for the induction of cellular defence machinery, including anti-oxidant enzymes. Various studies have used electrolytes leakage test (Ismail *et al.,* 1997; Kumari *et al*., 2009) for verifying the membrane damage of the cell in different abiotic stresses. Fokar *et al.,* 1998 had reported about the strong positive connection across genotypes between grain weight per spike and stability of the cell membrane as a heat tolerance measure. The membrane stability however may be a small portion of the various properties and processes that are associated for providing tolerance to stress during maturation (Assad and Paulsen 2002). As we know that membrane is the first barrier of defense having various heat responsive sensors which activates defense mechanism in advance against heat stress, so we can say that the integrity of the cell membrane is one of the important parameter for providing heat stress tolerance to the crop against heat stress.

From the present study It is concluded that the percentage conductivity of HDR 77 was more *i.e.* highly susceptible or least resistant variety and least percentage conductivity was observed in HD 2687 *i.e.* least susceptible or highly resistant variety. The membrane stability index (M.S.I.) of HDR 77 was less and highest membrane stability index was observed in HD 2687.

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Table 1. List of genotypes used for characterisation

SI. no.	Name of cultivar	Developed by Centre	Parentage	Area of adoption	Production conditions
1	PBW 373	P.A.U. LUDHIANA	ND/VG9144//KAL/BB/3/YCO" S"/4/VEE#5 "S"	NWPZ	LS, IR
2	HD 2967	I.A.R.I. N.DELHI	ALD/COC//URES/HD2160M/HD2278	NWPZ	TS, IR
3	HDR77	N.DELHI	PARTIZANKA /`HD 2204 //HD2204	NEPZ	LS, RF
4	Raj 4037	Rajasthan agricultural university DURGAPURA	DL 788-2/RAJ 3717	PZ	TS, LS, IR
5	HD2385	I.A.R.I. N.DELHI	HI-687 /HD-2268	FEZ	LS, IR
6	RSP 561	SKUAST-J	HD2637//Ae. *crassa*//HD2687		TS, LS, IR
7	HS 365	IARI regional station SHIMLA	HS 207 /SONALIKA	NHZ, HIGH ALT.	TS, RF
8	HD 2864	I.A.R.I. N.DELHI	DL 509-2/ DL 377-8	CZ	LS, IR
9	PBW 175	P.A.U. LUDHIANA	HD 2160 /WG 1025	NWPZ	TS, RF
10	JAUW 584	SKUAST-J	PDW233/Ae. *crassa*/ PBW343		TS, IR
11	HD2687	I.A.R.I. N.DELHI	CPAN2009 / HD 2329	NWPZ	TS, IR
12	HW 2045	I.A.R.I. regional station WELLINGTON (Tamil Nadu)	HD 2402 *6 /SUNSTAR *6 /C-80-1	NEPZ	LS, IR

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Table 2. Genotypes representing average, percentage conductivity and membrane stability index (MSI)

Genotypes	Average electrolyte leakage			Conductivity (%)	Membrane Stability Index
	Control (R.T.) (µs/cm)	45°C (µs/cm)	100°C (µs/cm)		
PBW 373	23.2	43	827	5.19	94.9%
HD 2967	25.6	36.1	625	5.78	94.3%
HDR77	22.7	41.1	582	7.06	93%
Raj 4037	24.9	33	719	4.59	95.5%
HD2385	24.4	28.1	948	2.96	97.1%
RSP 561	24.9	29.3	833	3.52	96.5%
HS 365	30.9	31.4	657	4.78	95.3%
HD 2864	26.9	54.9	931	5.90	94.2%
PBW 175	20.6	24.2	643	3.76	96.3%
JAUW 584	28.4	34.1	771	4.42	95.6%

Table 3. Analysis of Variance for electrolyte leakage

Source of variation	Degrees of Freedom (df)	Electrolyte leakage at R.T.	Electrolyte leakage at 45ºc	Electrolyte leakage at 100ºc
		Mean sum square		
Genotype	11	24.647**	235.78**	53705.23**
Replication	2	59.263**	25.95	1313.07**
Error	22	4.0984596	13.28	201.23
Critical difference		3.42	6.17	24.02
Coefficient of variance		8.15	10.66	1.82

** significant at 1 % level of significance