Indian Journal of Science and Technology, Vol 9(31), DOI: 10.17485/ijst/2016/v9i31/50284, August 2016
ISSN (Print) : 0974-6846
ISSN (Online) : 0974-5645

Evaluation of Seedling Growth and MDA Content of Wheat Genotypes in Relation to Heat Tolerance

Amritpal Kaur Sanghera* and S.K. Thind

Depatment of Botany, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana, India; amritpalkaursanghera@yahoo.in

Abstract

Forty wheat genotypes were grown in petriplates at different temperature treatments (25±2, 35±2 and 40±2˚C) to study the effect of heat stress on germination, growth rate and Malondialdehyde content (MDA). Vigor index and Relative Growth Rate (RGR) showed significant reduction with increasing temperature treatments (25±2 to 40±2˚C). Heat tolerant genotypes had higher values of vigor index and relative growth rate than heat sensitive genotypes. Whereas the MDA content of seedling at 35±2 and 40±2 ºC were higher as compared to those at 25 ºC. The increments of MDA content from 25 to 40 ºC were significant for all wheat genotypes. Heat sensitive genotypes accumulated more MDA content due to enhanced lipid peroxidation of membranes than tolerant and moderately tolerant genotypes. The seedling MDA content at higher temperature treatments also showed significant negative correlation with membrane thermostability (r= -0.617 and -0.861 at 35 and 40 ºC respectively) across the 40 wheat genotypes indicating that wheat genotypes with lower MDA content tended to show greater membrane thermotolerance.

Keywords: Heat Stress, MDA, RGR, Vigor Index, Wheat

1. Introduction

Wheat is the one of the important cereal crop in India. However, with rise in temperature around the globe, wheat may be exposed to greater heat stress[1]. Heat stress drastically affects both biochemical and physiological aspects of plant[2]. Plants are very sensitive towards heat stress and different plant processes can be irreversibly damaged by heat. Temperature is an important factor for wheat germination, because it influences not only the rate of water but also other substrates required for growth and development of seedling. Temperature plays an important role in germination because it affects the water absorption and nutrient supply required for normal growth and development of plant[3]. Higher temperature during germination affects membrane stability which enhances leakage of electrolytes and other macro-molecules in membrane and directly affects the germination and seedling vigor[4]. Heat stress damages the membrane integrity that ultimately causing leakage of ions from the cells. Damage caused by stress was estimated by electrolyte leakage of solute from leaf tissue after a heat shock[5].

Lipids are important structural elements of the cell which also play important role in permeability of membranes. So, it's very important to maintain these molecules for the proper functioning of membranes[6]. High temperature stress enhances the accumulation of Reactive Oxygen Species (ROS) which causes an oxidative stress[7]. The generation of these reactive oxygen species is considered as a key event under different types of stresses. It leads to the oxidative destruction of cells because of their highly reactive nature. ROSs produced due to temperature stress results in Lipid Peroxidation (LP) of membranes and also affects the functioning and inactivation of different types of enzymes inside the cell[8]. In membranes, peroxidation of unsaturated fatty acids results in cell membrane damage and MDA is produced as a result of peroxidation of lipids[9]. The stability of cell membranes increases with decrease in unsaturation of lipids[10]. So, peroxidation of

*Author for correspondence

lipids is good indicator of oxidative stress in cell membrane. Therefore, the total damage to cell after stress was tested by measuring the rate ion movement into and out of the cell membranes. In the present study, the effect of heat (35 and 45 °C) stress on the germination, growth of seedling and on biochemical parameters (Membrane thermotolerance and MDA concentration) of wheat seedlings was studied.

2. Materials and Methods

The study was conducted in the laboratories of Punjab Agricultural University, Ludhiana. A set of germplasm consisting of various lines under the different trial names, QTLs POPMAP-I to QTLs POPMAP-XX, PT CAN II sq-I to PT CAN II sq-XIV, CSIRO GCP-I and CSIRO GCP-II assembled by CIMMYT under the Generation Challenge Program were taken for the experiment. Four cultivated genotypes were also included, namely C306, PBW 343, PBW 621 and HD 2967. These genotypes were grouped into three categories on the basis of membrane thermosensitivity tests in previous experiments. Genotypes of the group I was termed as tolerant, Group II as susceptible and Group III was found as moderately tolerant on the basis of their membrane thermotolerance. In this experiment, we studied the effect of heat shock on germination, growth of seedling and lipid peroxidation of membrane (MDA content). To evaluate the effect of heat stress, seeds were germinated in petriplates and placed in an incubator with a temperature of 25±2˚C with the optimum level of water. Heat shock was given by placing the petriplates in an incubator with the temperature of 35±2˚C and 40±2˚C and the following observations were recorded:

1. Evaluation of germination vigor index: Seeds were germinated in growth chamber at 25±2ºC after sterilizing them with hypochloride sodium. Percentage of germination was determined 2 days after sowing. When the radicle emerged from the testa, seeds were considered germinated and their vigor index was calculated.

Vigour index = Vigour index was calculated as suggested by Abdul Baki and Anderson[11].

Vigour index = Germination (%) × Hypocotyl length (cm)

2. Relative growth rate (RGR): RGR was calculated by measuring the dry weight of wheat seedling at 4 and 8 days after germination.

$$RGR = \frac{\ln W_2 - \ln W_1}{T_2 - T_1}$$

Where W_1 is the dry weight at first sampling and W_2 is the dry weight at second sampling. T_2-T_1 is the time interval between two samplings

3. Malondialdehyde content[12]
4. Membrane thermostability[13]
5. Statistical analysis: Mean of three independent trails is given in tables. Level of significance ($p < 0.05$) was determined by using one-way ANOVA test to identify significant differences between the groups.

3. Results and Discussion

Vigor index of wheat seeds are presented in Figure1. Results showed that germination characteristics were strongly influenced by the high temperature treatments. There was significant decrease in germination and vigor index with increasing temperature treatments. Increase in temperature from 25°±2ºC to 45°±2ºC led to 67 % reduction in vigor index. Heat tolerant genotypes showed higher % germination and vigor index as compared to heat sensitive genotypes. Maximum values of vigor index were found in QTLs POPS MAP-IV and HD 2967. In other studies, lower germination of wheat genotypes was also found due to heat stress[14]. Germination is one of important and vulnerable period of plants life cycle. The state of seedlings and plants growth is determined by germination[15]. So reduction in it because of high temperature conditions could be attributed to damage of seeds during development and maturation. Optimum temperature is the main requirement for higher seed vigor index in wheat[2].

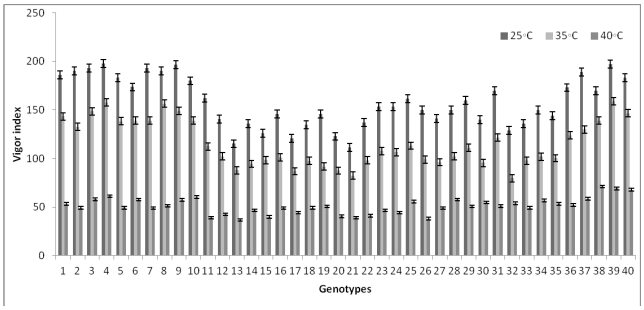

Figure 1. Vigor index of 40 wheat genotypes at seedling stage under normal (25±2ºC) and higher temperature conditions (35±2ºC and 40±2ºC).

Table 1. MDA (µmoles /g fresh weight) of 40 wheat genotypes at seedling stage under normal (25±2°C) and higher temperature conditions (35±2°C and 40±2°C).

Serial Number	GENOTYPE	25±2°C	35±2°C	40±2°C
GROUP I	QTLs POPS MAP-I	0.624±0.007	0.782±0.041	0.810±0.035
	QTLs POPS MAP-II	0.966±0.058	1.066±0.011	1.156±0.054
	QTLs POPS MAP-III	0.748±0.072	0.698±0.017	0.811±0.070
	QTLs POPS MAP-IV	0.690±0.017	0.822±0.027	0.973±0.034
	QTLs POPS MAP-V	0.756±0.089	0.700±0.047	1.272±0.028
	QTLs POPS MAP-VI	0.574±0.014	0.608±0.058	0.887±0.007
	QTLs POPS MAP-VII	0.799±0.034	0.913±0.016	1.010±0.040
	QTLs POPS MAP-VIII	0.824±0.010	1.364±0.025	1.033±0.030
	QTLs POPS MAP-IX	0.640±0.055	1.037±0.072	1.298±0.058
	QTLs POPS MAP-X	0.713±0.016	0.811±0.010	1.073±0.089
GROUP II	QTLs POPS MAP-XI	0.824±0.088	1.096±0.035	1.535±0.099
	QTLs POPS MAP-XII	0.807±0.083	1.916±0.035	2.655±0.054
	QTLs POPS MAP-XIII	0.824±0.037	1.981±0.070	2.099±0.019
	QTLs POPS MAP-XIV	0.890±0.030	1.639±0.034	2.373±0.014
	QTLs POPS MAP-XV	0.982±0.017	1.016±0.028	1.536±0.082
	QTLs POPS MAP-XVI	0.623±0.072	1.273±0.007	2.196±0.035
	QTLs POPS MAP-XVII	.820±0.010	1.648±0.040	2.094±0.093
	QTLs POPS MAP-XVIII	0.957±0.035	1.038±0.030	1.811±0.006
	QTLs POPS MAP-XIX	0.724±0.053	1.089±0.058	1.962±0.018
	QTLs POPS MAP-XX	.907±0.642	1.400±0.089	1.886±0.042
GROUP III	PT CAN II sq-I	.805±0.047	1.189±0.078	1.772±0.342
	PT CAN II sq-II	0.633±0.041	1.633±0.055	1.730±0.068
	PT CAN II sq-III	0.907±0.011	1.097±0.072	1.573±0.088
	PT CAN II sq-IV	0.857±0.017	1.397±0.035	1.650±0.095
	PT CAN II sq-V	0.602±0.027	1.164±0.094	1.423±0.068
	PT CAN II sq-VI	0.724±0.047	1.565±0.006	1.231±0.088
	PT CAN II sq-VII	0.738±0.058	1.256±0.018	1.835±0.083
	PT CAN II sq-VIII	0.714±0.016	1.633±0.021	1.672±0.037
	PT CAN II sq-IX	0.849±0.025	1.312±0.095	1.622±0.068
	PT CAN II sq-X	0.704±0.034	1.367±0.068	1.503±0.041
	PT CAN II sq-XI	0.641±0.028	1.149±0.088	1.635±0.049
	PT CAN II sq-XII	0.690±0.007	1.490±0.083	1.273±0.078
	PT CAN II sq-XIII	0.733±0.040	1.038±0.037	1.310±0.055
	PT CAN II sq-XIV	0.773±0.030	1.148±0.068	1.286±0.072
	CSIRO GCP-I	0.940±0.058	1.615±0.041	1.736±0.035
	CSIRO GCP-II	0.711±0.089	1.057±0.099	1.234±0.094
GROUP IV	C306	0.808±0.072	0.877±0.069	1.042±0.006
	PBW 621	0.807±0.035	0.967±0.089	0.976±0.018
	HD 2967	0.678±0.078	0.738±0.014	0.898±0.042
	PBW 343	0.883±0.055	0.967±0.019	1.109±0.072
MEAN		0.786	1.189	1.474
LSD(0.05)		0.390	0.167	0.241
*CV%		29.54	8.64	10.15

* Coefficient of Variation (%)

In wheat high temperature stress during seedling stage inhibits the leaf and root growth of plant, which directly affected the relative growth rate (RGR) of seedling (Figure 2). Significant reduction was observed in RGR of wheat genotypes with increasing temperature treatments. This trait showed 31 % reduction due to heat shock. Heat tolerant genotypes had better growth rate as compared to sensitive genotypes. Reduction in seedling dry weight was a result of reduction in seed reserve mobilization, mainly at severe temperature stress[16]. Significant reduction in RGR of wheat genotypes due to temperature stress was also found earlier[17].

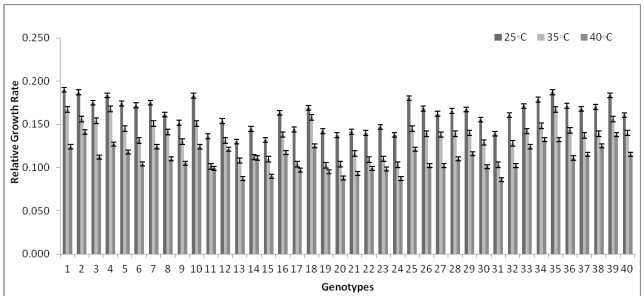

Figure 2. RGR of 40 wheat genotypes at seedling stage under normal (25±2ºC) and higher temperature conditions (35±2ºC and 40±2ºC).

The effect of heat stress on malondialdehyde content is shown in Table 1. Significantly higher MDA content was recorded in heat-stressed genotypes *i.e* MDA content of genotypes grown at 35 and 40 ºC were higher compared to those at 25 ºC. These increments of MDA content were significant for all wheat genotypes from 25 to 40 ºC temperature treatments. On an average, mean MDA content was 0.786 µmol/gm.F.W in seeds at normal temperature treatment, whereas heat treated seeds at 35 and 40 ºC had higher MDA content *i.e.* 1.189 and 1.474 µmol/gm.F.W respectively. At 25 ºC, the MDA content was same in heat sensitive and heat tolerant groups, but the higher MDA content was found in the heat sensitive group ranged between 1.03-1.98 µmol/gm.F.W and 1.5 – 2.65 µmol/gm.F.W at 35 and 40 ºC respectively as compared to tolerant (0.608-1.364 µmol/gm.F.W and 0.810 – 1.298 µmol/gm.F.W at 35 and 40 ºC respectively) and moderately tolerant genotypes (1.03-1.633 µmol/gm.F.W and 1.234 – 1.835 µmol/gm.F.W at 35 and 40 ºC respectively). Check varieties, tolerant and moderately tolerant genotypes showed the less amount of MDA content in stressed leaf tissue as compared to sensitive genotypes because they had tendency to control the lipid peroxidation under stressed conditions. Results from other studies also found increase in MDA content in heat sensitive genotypes of wheat[18]. In response to a heat stress all wheat genotypes accumulated more amount of MDA content due to lipid peroxidation of membranes that clearly indicating an occurrence of oxidative stress under the effect of a high temperature. When temperature conditions are favorable plants tends to maintain a balance between producing and scavenging reactive oxygen species. Heat stress may disturb this balance and promote lipid peroxidation, which ultimately induces the MDA accumulation[19].

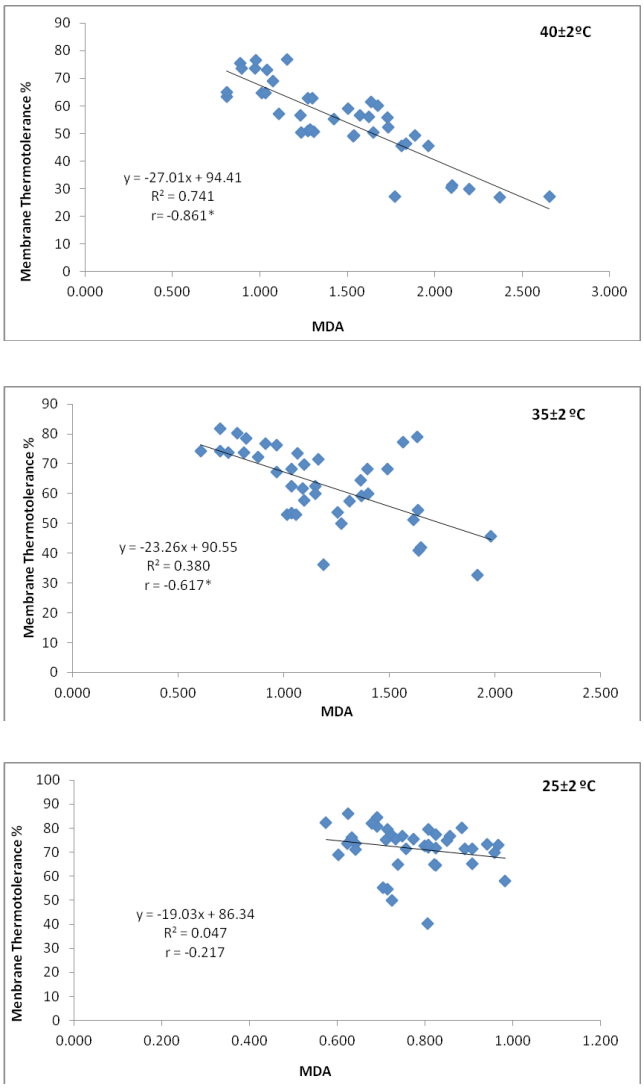

Figure 3. Relationship between membrane thermotolerance (%) and MDA content at seedling stage under normal (25±2ºC) and higher temperature conditions (35±2ºC and 40±2ºC).

The seedling MDA content at higher temperature treatments and membrane thermotolerance percentage maintained a significant negative correlation (r= -0.617 and -0.861 at 35 and 40 °C respectively) across the 40 wheat genotypes indicating that wheat genotypes with lower MDA content tended to show greater membrane thermotolerance (Figure 3). This association was non-significant (r= 0.217) at 25 °C temperature conditions.

4. Conclusion

High temperature stress reduced the germination and growth rate of wheat seedling whereas MDA content increased correspondingly with increasing temperature from 25 to 40 °C. Heat sensitive genotypes accumulated more MDA content due to enhanced lipid peroxidation of membranes than tolerant genotypes. So MDA content could be used as selection criterion to screen wheat genotypes for thermotolerance.

5. References

1. Hossain A, Teixeira da Silva JA. Phenology, growth and yield of three wheat (triticum aestivum l.) varieties as affected by high temperature stress. *Not Sci Biol*. 2012; 4:97-109.
2. Sikder S, Paul NK. Evaluation of heat tolerance of Wheat cultivars through physiological approaches. *Thai J Agric Sci*. 2010; 43:251- 258.
3. Essemine J, Ammar S, Jbir N, Bouzid S. Sensitivity of two Wheat species seeds (Triticum durum, variety Karim and Triticum aestivum, Variety Salambo) to heat constraint during germination. *Pak J Biol Sci*. 2002; 10:3762-3768.
4. Girelberg A, Horowitz M, Poljakoff-Mayer A. Solute leakage from *Solanum Nigram* (L.) seeds exposed to high temperature during imbibation. *J Exp Bot*. 1984; 35:1754-1763.
5. Saadalla MM, Quick JS, Shanahan JF. Heat tolerance in winter wheat: II. Membrane thermostability and field performance. *Crop Sci*. 1990; 30:1248-1251.
6. Liljenberg CS. The effects of water deficit stress on plant membrane lipids. *Prog Lipid Res*. 1992; 31:335-343.
7. Wormuth D, Heiber I, Shaikali J, Kandlbinder A, Baier M, Dietz KJ. Redox regulation and antioxidative defence in Arabidopsis leaves viewed from a systems biology perspective. *Biotech*. 2007; 129:229-248.
8. Blokhina O. Anoxia and Oxidative Stress: Lipid Peroxidation, Antioxidant Status and Mitochondrial Functions in Plant. Academic dissertation, Helsinki, 2000; p. 79.
9. Halliwel B, Gutteridge JMC. Free radicals in biology and medicine. 2nd ed. Claredon Press, Oxford, UK; 1989.
10. Costa Oliveira MS. Study of lipid composition and effect of water stress in dwarf cashew (Anacardium occidentale L.) leaf membranes. Master's thesis, Federal University of Ceará, Fortaleza, Brazil, 2001.
11. Abdul-Baki AA, Anderson JD. Vigor determination in soybean seed by multiple criteria. *Crop Sci*. 1973; 13:630-633.
12. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979; 95:351-58.
13. Shanahan JF, Edwards IB, Quick JS, Fenwick. Membrane thermostability and heat tolerance of spring wheat. *Crop Sci*. 1990; 30:247-51.
14. Hasan MA, Ahmed JU, Hossain T, Mian MAK, Haque MM. Evaluation of the physiological quality of wheat seed as influenced by high parent plant growth temperature. *J. Crop. Sci. Biotech*. 2013; 16: 69-74.
15. Gheraibia H, Souiki L, Bennoua S, Djebar MR. Comparative Study of the Biochemical and Physiological Mechanisms of Two Varieties of Durum Wheat (Triticum durum L.) Subject to Salt Stress. Ind. J. Sci. and Tech. 2016; 9(7). DOI: 10.17485/ijst/2016/v9i7/84670.
16. Essemine J, Ammar S, Bouzid S. Impact of heat stress on germination and growth in higher plants: physiological, biochemical and molecular repercussions and mechanism of defense. *J. Biol. Sci*. 2010; 10:565-572.
17. Almeselmani M, Deshmukh PS. Effect of high temperature stress on physiological and yield parameters of some wheat genotypes recommended for irrigated and rainfed condition. *Jordan J. Agric. Sci*. 2012; 8:66-78.
18. Dhyani K, Ansari MW, Rao RS, Verma, Shukla A, Tuteja N. Comparative physiological response of wheat genotypes under terminal heat stress. *Plant Signal Behav*. 2013; 8:e24564; http://dx.doi.org/10.4161/psb.24564.
19. Savicka M, Skute N. Effects of high temperature on malondialdehyde content, superoxide production and growth changes in wheat seedlings (*Triticum aestivum* L.). *Ekologija*. 2010; 56:26-33.