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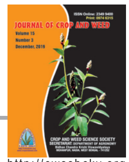

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Effects of heat and drought stress on yield and physiological traits in wheat (*Triticum aestivum* L.)

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ABSTRACT

Heat and drought stress are major wheat production constrains in South Asia. The present experiment was conducted with 22 wheat genotypes under optimum, drought, and heat stress conditions to study the effect of stress on yield and its contributing traits. Significant genetic variations were recorded for all the traits under optimum and both the stress conditions. All the traits were affected under stress among them grain yield was affected most. An average yield reduction of 53.4% was recorded under drought stress. Thousand-kernel weight (TKW), canopy temperature (CT), days to maturity (DM) affected maximum under heat stress. Drought stress drastically reduced membrane stability index (MSI), relative water content (RWC), root length (RL), dry root weight (DRW) and fresh root weight (FRW). RL was increased under drought stress but decreased under heat stress. A significant positive association of grain yield with TKW under heat and drought stress and with RWC under drought stress explained their utilization for selection of genotypes. The genotypes DWR 13-1, SSN 1330-2-2, DWR 37-2 and DWR 2-4 were identified as heat and drought tolerant which may be further used in the breeding program.

Keywords : Drought stress, heat stress, membrane stability index, relative water content, wheat

India is the second-largest producer of wheat with the annual production of 102.19 million tonnes from 29.14 million hectare areas and productivity of 35.07 q/ha in the year 2018-19 (Directorate of Economics and Statistics, 2018-19). North Eastern Plain Zone (NEPZ) of India covers nearly 10 million hectare wheat-growing areas where rice-wheat cropping system is most prominent. Wheat sowing is delayed due to the late harvesting of rice, as a result the crop faces terminal heat stress at the post-anthesis period (Joshi *et al.*, 2007). The optimum temperature during anthesis and grain filling stage is proposed to be 12ºC and 22ºC, respectively (Mullarkey and Jones 2000; Tewolde *et al.*, 2006). Around 40% of the total wheat-growing areas in the world including South Asia are affected by terminal heat stress (Reynolds *et al.*, 2001 and Joshi *et al.*, 2007).

Besides, shortage of irrigation water drastically influencing the yield potentiality of wheat varieties. Increase trend of drought stress in central and Indo-Gangetic plains of India is challenging in wheat production indicating vulnerability of food security (Zhang *et al.*, 2017). Drought stress can cause yield reduction up to 92% depending upon the stress and crop growth (Farooq *et al.*, 2014 and Semenov *et al.*, 2014). A meta-analysis based on previous publications estimated an average yield loss of 27.5% due to drought (Zhang *et al.*, 2018). Drought as well as heat stress adversely affect plant growth, physiological status, reproduction and yield (Mittler *et al.*, 2011). Several yield-attributing traits including thousand kernel weight, chlorophyll content and membrane stability index were found to be associated with grain yield under heat or drought stress (Bansal *et al.*, 2016 and Rehman *et al.*, 2016). Limited experiments have been undertaken to study the effect of heat and drought stress at the same time on wheat crop. One report studied the effect of heat and drought stress on growth and yield attributing parameters in wheat (Qaseem *et al.*, 2019), where yield was affected most in combined heat and drought stress followed by heat stress and drought stress only. It is necessary to study further the effect of heat and drought stress on yield and its attributing traits for their utilization in the breeding programme. Several indices have been proposed for the selection of genotypes under stress condition (Fischer and Maurer, 1978 and Hossain *et al.*, 1990). Mau *et al.* (2019) suggested the use of stress tolerance index, stress susceptible index, mean productivity for selection of drought tolerance genotypes in rice. Stress susceptible index have been used by many researchers to select genotypes for drought and heat tolerance (Bennani *et al*., 2017; Ali and El-Sadek, 2016; Grzesiak *et al.*, 2019). The present experiment was planned to study the genetic analysis of physiological traits under heat and drought stress and their relationship with yield.

MATERIALS AND METHODS

Twenty-two wheat genotypes including released varieties and advanced breeding lines and genotypes selected on the basis of their agronomic performances

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from different national and international nurseries were evaluated in the farm of Bihar Agricultural University, Sabour, Bhagalpur, India (Table 4). Among these genotypes BRW 3723 and DBW 14 were used as check for drought and heat tolerant, respectively and Sonalika as susceptible check. The experiments were planned in a randomized complete block design with three replications at optimum, drought, and heat stress conditions in 4 rows plot with 2 m row length and 20 cm row to row spacing for each genotype. Experiments under optimum and drought stress were sown on 26th November, 2018 in the field and under rainout shelter, respectively. The experiment for heat stress was sown on 26th December, 2018 in the field to expose the crop at high temperature than the optimum (23ºC) during anthesis to post-anthesis stages of crop growth. Five irrigations were scheduled at crown root initiation (CRI), maximum tillering, booting (Zadoks' growth stage, GS 45), milk development (GS 73) and dough development (GS 85) in both optimum and heat stress condition. One irrigation was given in drought stress experiment at crown root initiation (CRI) stage to create moisture stress at the terminal stage of crop growth particularly at anthesis and post-anthesis period. Recommended agronomic practices and plant protection measures were undertaken time to time to raise a healthy crop. Soil moisture percentage was recorded from all the experiments as a difference between fresh weight and oven-dry weight of soil sample taken at 5 cm depth from the top in each replication. The soil moisture level was measured at CRI, anthesis and physiological maturity stages of crop growth. Records on precipitation and temperature for each day during the crop growth period were recorded at the university weather station.

Observations were recorded for days to maturity (DM), thousand kernel weight (TKW), membrane stability index (MSI), canopy temperature (CT), SPAD value as a measure of leaf chlorophyll status, relative water content (RWC), root length (RL), fresh root weight (FRW), dry root weight (DRW) and grain yield (GY). For measuring the root traits, plants were uprooted along with the adjoining soils from the field at anthesis stage. The soil adjoining to the roots was cut in a block to a size of 40 cm depth and 15 cm diameter from the base. The soil was washed off in a bucket using a mesh of 0.7 mm. After that, the samples were washed gently in tap water to remove the excess soil. During washing, care was taken to avoid root breakage. The samples were wiped with tissue paper to remove excess water present on the root. After cleaning properly root length and fresh root weight were recorded. Dry root weight was measured after drying of the root sample at 72ºC in the oven for 48 hrs. Physiological parameters like SPAD reading as a measure of chlorophyll content was recorded using SPAD meter (SPAD 502, Minolta, Japan) at anthesis. The data was recorded as an average of five flag leaves selected from each genotype. For each leaf, three readings were taken at the top, middle, and bottom of the leaf blade and average of all three was considered for one leaf. Canopy temperature (CT) was taken using hand handled infrared thermometer (FLUKE 62 Mini IR thermometer, China) at post-flowering stage during bright sunny days in between 1-2 pm. CT recording was avoided immediate after irrigations. For each genotype average of three measurements was taken.

Relative water content was measured at anthesis by taking an average of five flag leaves from each genotype. The collected leaf samples were cut from both ends and weighed to record the fresh weight (FW). The leaves were hydrated in double-distilled water for 4 hours and the samples were again weighed to record the turgid weight (TW). Then the leaves were dried at 72^0C for 48 hours and dry weight (DW) was measured. RWC (%) was calculated based on the following formula.

$$RWC = \{(FW-DW)/(TW-DW)\}\times 100$$

Membrane stability index was measured at anthesis as an average three flag leaves from each genotype. The collected leaf samples were dipped in 50 ml double-distilled water in separate test tubes and kept overnight. After 24 hours, electrolytes leakage was measured by reading electrical conductivity (EC) using EC meter. Then the whole samples were autoclaved for 15 minutes and again EC was measured (EC,). MSI (%) was calculated using the following formula.

MSI = {1-(EC• /EC,)}×100

The stress tolerance index was calculated as per Fisher and Maurer (1978) as follows

$$\text{Heat susceptibility index (HSI)} = (1-Y_s/Y_p)/(1-\frac{\overline{Ys}}{\overline{Yp}})$$

Where, Ys and Yp being the yield of genotypes evaluated under heat stress and non-stress conditions and the mean yield of overall genotypes were evaluated under heat stress and non-stress conditions, respectively.

RESULTS AND DISCUSSION

Significant genetic variation for all the traits evaluated under optimum, drought and heat stress revealed the presence of genotypic difference (Table 1a, 1b and 1c). Weekly temperature recorded during the crop growing period revealed maximum temperature crossed the optimum level during first to second week of March (Figure 1). At that time most of the genotypes sown under heat stress experiment were at anthesis (GS 69) to early milk stage (GS 73) while the genotypes under the optimum experiment were at hard dough (GS 87) to physiological maturity stage depending upon the maturity duration. Soil moisture status of the

Table 1a. Analysis of variance for the traits evaluated under optimum condition

	df	DM	TKW (g)	MSI	CT (ºC)	SPAD value	RWC (%)	RL (cm)	FRW (g)	DRW (g)	GY (q/ha)
Genotype	**21**	16.47	152.10	90.05	12.37	2.28	16.32	0.01	0.22	0.17	126.34
Replication	**2**	49.32**	32.24**	179.72**	1.71**	19.53**	55.71**	14.10**	9.10**	1.80**	74.97*
Error	**42**	5.10	2.18	16.53	0.56	7.17	8.63	1.85	0.52	0.11	35.78

*&** indicates the p value at 0.05 and 0.01

Table 1b. Analysis of variance for the traits evaluated under drought stress condition

	df	DM	TKW (g)	MSI (%)	CT (ºC)	SPAD value	RWC (%)	RL (cm)	FRW (g)	DRW (g)	GY (q/ha)
Genotype	**21**	7.68	12.76	11.08	0.35	7.25	44.21	2.97	0.22	0.04	27.47
Replication	**2**	81.2**	12.28**	138.4**	2.52**	11.96 *	66.99**	10.21**	1.36**	0.54**	104.22**
Error	**42**	5.08	3.88	9.01	1.03	5.78	15.69	2.49	0.16	0.06	7.05

*&** indicates the p value at 0.05 and 0.01

Table 1c. Analysis of variance for the traits evaluated under heat stress condition

	df	DM	TKW (g)	MSI (%)	CT (ºC)	SPAD value	RWC (%)	RL (cm)	FRW (g)	DRW (g)	GY (q/ha)
Genotype	**21**	95.09	248.35	16.7	54.55	27.37	16.43	9.35	1.87	0.1	18.89
Replication	**2**	41.8**	61.70**	97.56**	1.08*	26.99**	16.32*	7.5*	7.32**	1.52**	51.74**
Error	**42**	11.34	13.41	19.86	0.48	7.76	7.35	3.31	0.5	0.14	5.46

*&** indicates the p value at 0.05 and 0.01

experimental plots at CRI, anthesis, and at physiological maturity revealed that the moisture was drastically reduced at anthesis followed by physiological maturity under drought stress (Figure 2).

All the traits were affected by the heat and drought stress compared to optimum; however, maximum reduction in grain yield (53.42%) was recorded under drought stress (Table 2). Under heat stress, an average yield reduction of 40% was recorded. Joshi *et al.* (2007) reported 45% yield reduction in wheat under late sown condition. In another study, up to 35% yield reduction in wheat was reported due to high temperature at anthesis (Talukder *et al.*, 2014). Low heritability for grain yield under optimum condition was observed; however, high heritability and high genetic advance under stress conditions indicate the possibilities of selection of suitable genotypes.

Stress at terminal stages of crop growth imposes adverse effect on crop maturity duration. We recorded a reduction in maturity duration under both the stresses than the optimum growing condition. However, the effect of heat stress on DM was higher than the drought resulting in reduction of an average of 14 days duration than the optimum. Heritability of DM was high under optimum, moderate under drought stress and low under heat stress. It can be revealed that increased temperature during grain filling stages imposed rapid premature drying of crops. As a result of shortening grain filling duration under heat stress affected the TKW and GY. An average of 7.01 g TKW were reduced under heat-stress than the optimum. TKW is suggested for use in selection for genotypes under stress conditions (Mondal *et al.*, 2016 and Rosyara *et al.*, 2010). The promising entries found under heat stress were DWR 13-1; DWR 3-2; DWR 35-2-1; DWR 2-4; DWR 22-4. A significant positive association of TKW was found with grain yield under drought stress (Table 3). BRW 3723 and C 306 were found to be tolerant to drought stress with higher GY and TKW. Both the genotypes were released for growing in rainfed areas. Another promising genotype under drought stress condition was DWR 13-1.

Roots play an important role in abiotic stress, particularly in drought stress. High variability for root length and dry root weight were reported under drought stress (Bansal *et al.*, 2016). However, the effect of above-ground heat stress on root traits was not studied in detail in wheat. In the present experiment, RL was increased under drought stress and decreased in heat stress than the optimum. FRW and DRW were reduced under drought and heat stress but the reduction was maximum in drought stress. Reduction of fresh and dry root weight in wheat under drought stress condition was observed by Faisal *et al.* (2017). Surprisingly, no association was found for any of the root traits with grain yield both under optimum and stress conditions.

Table 2. Average measures, heritability and genetic advance of each trait evaluated under optimum, drought and heat stress condition

Particulars	**DM**			**TKW (g)**			**MSI (%)**			**CT (ºC)**			**SPAD value**		
Growing condition	**Optimum**	**Drought Stress**	**Heat Stress**	**Optimum**	**Drought Stress**	**Heat Stress**	**Optimum**	**Drought Stress**	**Heat Stress**	**Optimum**	**Drought Stress**	**Heat Stress**	**Optimum**	**Drought Stress**	**Heat Stress**
Mean	125.35	120.59	109.5	38.18	35.01	31.17	47.21	39.28	42.14	26.2	29.63	31.77	48.11	50.19	41.55
Heritability (%)	74.3	83.3	47.2	82.2	41.9	54.6	76.7	82.7	56.6	40.7	32.3	29.4	36	26	45
Genetic advance	5.44	7.86	4.12	15.48	6.38	19.59	28.19	31.32	18.72	3.11	2.81	1.58	5.25	3.02	8.44
CV(%)	**1.8**	**1.87**	**3.07**	**3.86**	**5.63**	**11.75**	**8.61**	**7.64**	**10.58**	**2.85**	**3.41**	**2.18**	**5.56**	**4.79**	**6.7**
$CD_{0.05}$	**3.72**	**3.71**	**5.55**	**2.43**	**3.25**	**6.03**	**6.7**	**4.95**	**7.34**	**1.23**	**1.66**	**1.14**	**4.41**	**3.96**	**4.59**
Particulars	**RWC (%)**			**RL (cm)**			**FRW (g)**			**DRW (g)**			**GY (q/ha)**		
Growing condition	Irrigated	Drought Stress	Heat Stress	Irrigated	Drought Stress	Heat Stress	Irrigated	Drought Stress	Heat Stress	Irrigated	Drought Stress	Heat Stress	Irrigated	Drought Stress	Heat Stress
Mean	87.17	80.19	83.56	13.33	15.37	12.5	4.65	3.06	4.51	2.17	1.42	1.97	49.55	23.08	29.71
Heritability(%)	64.5	52.1	28.9	68.8	50.8	29.7	84.7	71.7	82.1	83.1	72.7	77	26.8	82.1	73.9
Genetic advance	7.52	7.67	2.29	25.89	15.31	10.6	68.99	36.09	62.44	64.78	49.51	62.1	7.77	46.03	23.4
CV(%)	**3.37**	**4.94**	**3.24**	**10.22**	**10.27**	**14.54**	**15.44**	**13.01**	**15.61**	**15.54**	**17.27**	**18.79**	**12.07**	**11.5**	**7.86**
$CD_{0.05}$	**4.84**	**6.53**	**4.47**	**2.24**	**2.6**	**3**	**1.18**	**0.66**	**1.16**	**0.56**	**0.4**	**0.61**	**9.85**	**4.37**	**3.84**

*&** indicates the p value at 0.05 and 0.01

Table 3. Pearson's correlation coefficient of the traits with grain yield per evaluated under optimum, drought and heat stress condition

	DM	**TKW**	**MSI**	**CT**	**SPAD value**	**RWC**	**RL**	**FRW**	**DRW**
Optimum	-0.19	0.46*	-0.08	-0.13	0.31	0.03	0.15	0.15	0.2
Drought stress	0.39	0.45*	0.05	-0.21	-0.09	0.42*	0.1	0.03	0.08
Heat stress	-0.04	-0.01	0.001	-0.14	0.02	-0.05	-0.26	0.16	0.12

*&** indicates the p value at 0.05 and 0.01

Table 4. Grain yield (q/ha) under optimum, drought and heat stress condition of the genotypes and their drought and heat susceptible index (Fischer and Maurer (1978)

Genotypes	Optimum	Drought stress	Heat stress	Drought susceptibility index (DSI)	Heat susceptibility index (HSI)
W×S 16-2	46.43±0.38	26.54±0.15	24.30±0.44	0.80	1.19
NHP 56-3	47.50±0.73	20.94±0.15	27.21±0.17	1.05	1.07
DWR 13-1	45.81±0.98	28.99±0.50	38.07±0.36	0.69	0.42
SSN 50-2	44.60±1.64	16.30±0.50	31.52±0.37	1.19	0.73
HPYT 430	38.58±1.47	18.06±0.36	29.79±1.21	1.00	0.57
HPYT 429	50.38±0.84	17.48±0.58	23.95±0.37	1.22	1.31
DBW 14	48.12±1.80	19.20±0.62	30.70±0.34	1.13	0.90
SAWYT 4	55.69±0.81	15.63±0.08	32.08±0.41	1.35	1.06
SSN 1330-2-2	51.04±1.59	27.61±0.60	31.02±0.21	0.86	0.98
DWR 37-2	44.76±1.18	22.27±0.20	34.70±0.45	0.94	0.56
BRW 3806	54.24±1.16	26.13±0.41	31.20±0.34	0.97	1.06
C 306	48.03±1.16	35.07±0.13	21.61±0.50	0.51	1.37
BAZ	51.34±0.69	19.09±0.39	27.71±0.71	1.18	1.15
HUW 468	44.21±1.55	19.15±0.53	29.01±0.55	1.06	0.86
HD 2967	55.25±2.67	26.90±0.23	32.48±0.50	0.96	1.03
BRW 3723	59.58±1.15	35.19±0.59	26.70±0.49	0.77	1.38
Sonalika	47.28±0.75	15.34±0.12	24.70±1.02	1.26	1.19
DWR 22-4	56.11±1.23	21.50±0.16	33.76±0.24	1.15	0.99
DWR 35-2-1	53.04±1.58	16.85±0.16	34.52±0.27	1.28	0.87
DWR 2-4	45.32±1.01	26.84±0.43	34.28±0.40	0.76	0.61
DBW 39	48.85±1.36	25.49±0.51	27.47±0.47	0.90	1.09
DWR 19-2	53.87±1.22	27.19±0.29	26.95±0.44	0.93	1.25

MSI and RWC were decreased under stress; however, the effect of drought stress was higher on MSI and RWC than the heat stress. An average of 8% RWC in the flag leaf was reduced in drought stress than the optimum. Similar finding is also reported in earlier studies (Soltys *et al.*, 2016 and Siddique *et al.*, 2000). Bansal *et al.* (2016) recorded 37.5% and 22.59% of reduction in MSI and RWC, respectively under drought stress. Heritability of RWC under drought and heat stress was reduced while heritability of MSI was increased in drought but reduced in heat stress than optimum condition. Significant association of RWC with grain yield under drought stress was observed indicating use of the traits in genotype selection.

An average of 8.29% increase in SPAD reading under drought stress while 13.63% decreased in heat stress than the optimum indicated that heat stress drastically reduced leaf chlorophyll status. The relationship of SPAD value with leaf chlorophyll, TKW and yield under stress was also reported in earlier studies (Shah *et al.*, 2017 and Rosyara *et al.*, 2010). Rosyara *et al.* (2010) suggested the use of SPAD reading for heat tolerant genotypes. High canopy temperature was recorded under heat stress than the optimum and drought stress. Heritability for CT was moderate and its association with grain yield was non-significant but negative in all three conditions. Positive association of canopy temperature depression with grain yield and thousand kernels weight was reported in wheat (Paliwal *et al.*, 2012). However, Pierre *et al.* (2010) reported a significantly negative correlation of CT with grain yield under drought stress.

Differential genotypic response was observed for drought and heat stress index. Grzesiak *et al.* (2019) suggested the use of drought susceptible index (DSI) for effective selection of genotypes. Under drought stress eleven genotypes were found to be moderately tolerant, among which C 306 showed maximum tolerance potential (Table 4). DWR 13-1 was identified as tolerant to heat stress. Besides, nine genotypes were identified as moderately tolerant to heat stress. The stress tolerance index has been effectively used for the selection of genotypes (Paliwal *et al.*, 2012, Pandey *et al.*, 2015). C 306 and BRW 3723 were better adapted to drought stress. C 306 was drastically affected by heat stress compared to drought stress. However, in previous study, C 306 was reported as tolerant to heat stress (Guha *et al.*, 2001). Besides, four genotypes DWR 13-1, SSN 1330-2-2, DWR 37-2 and DWR 2-4 were found to be better adapted to both the drought and heat stress

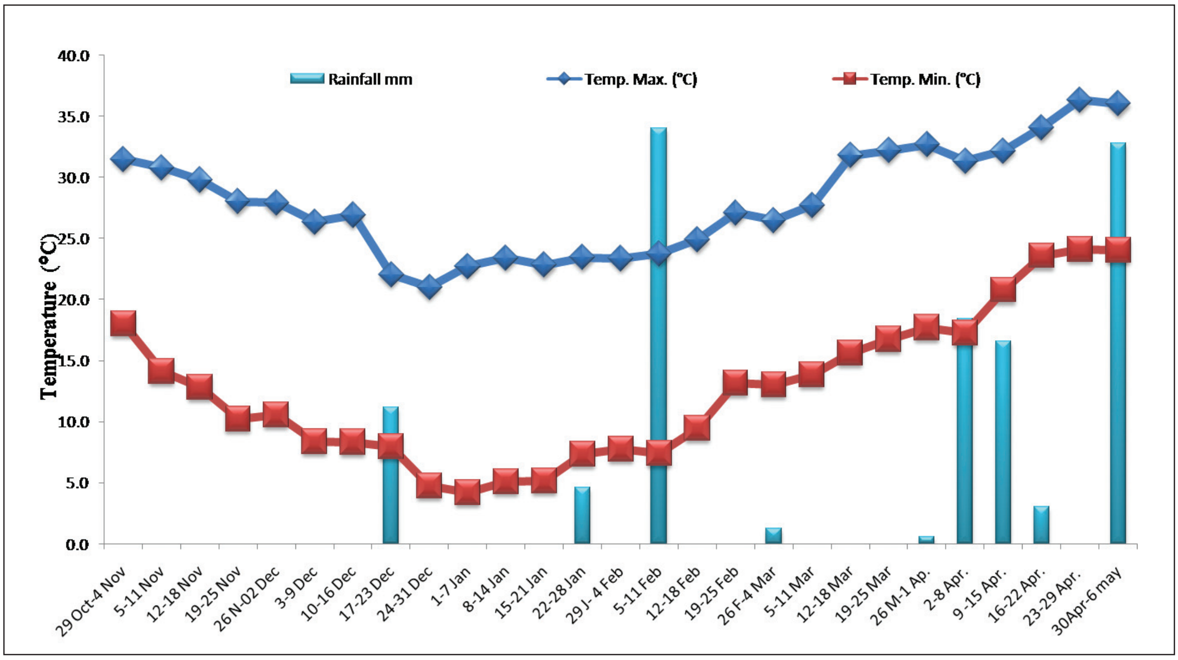

Fig. 1 : Weekly rainfall, maximum and minimum temperature during the crop growing season

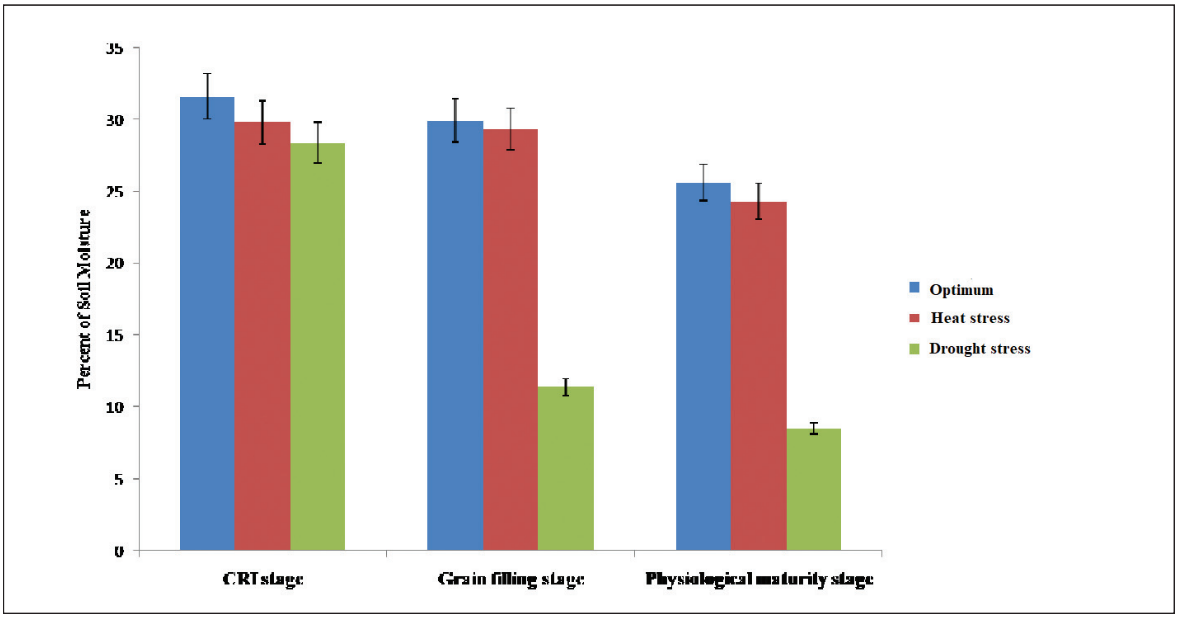

Fig. 2 : Soil moisture percentage at different growth stages under optimum, heat stress and drought stress condition

conditions. These genotypes may be used further for development of new cultivars.

In conclusion, grain yield was affected maximum under drought stress. RWC and MSI may be considered for screening of genotypes under drought stress. The genotypes, DWR 13-1, SSN 1330-2-2, DWR 37-2 and DWR 2-4 were identified as heat and drought tolerance which may be used further in the breeding program.

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