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EVALUATION OF CHLOROPHYLL CONTENT INDEX AND NORMALIZED DIFFERENCE VEGETATION INDEX AS INDICATORS FOR COMBINE EFFECTS OF DROUGHT AND HIGH TEMPERATURE IN BREAD WHEAT GENOTYPES

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ABSTRACT
Wheat (*Triticum aestivum* L.) a staple food crop, is of great commercial importance. Its production is restricted due to multiple environmental stresses. The aim of this study was to investigate the effect of high temperature, drought and their combination effect on biomass and yield in addition to the stress adaptive traits like canopy temperature (CT), chlorophyll content (SPAD) and normalized difference vegetative index (NDVI). This study was conducted during winter season of mid-November to April, 2015-16 and 2016-17 with eight selected wheat genotypes *viz*. AKAW-3717, C-306, DHTW-60, HD-2967, HTW-11, Kundan, WH-730 and WH-1105. In control (irrigated) and drought-stressed experiments, genotypes were sown at optimum planting date, while for heat-stressed experiment sowing date was delayed. Experiments were conducted in randomized complete block design with five replications. Chlorophyll provide an assessment of leaf nitrogen, an essential plant nutrient, due to the close relationship between leaf chlorophyll and leaf nitrogen. Combine effects of these stress dramatically increased canopy temperature and reduced chlorophyll content and normalized difference vegetation index in sensitive genotypes (WH-1105 and HD-2967) whereas in tolerant genotypes (DHTW-60 and C-306) higher SPAD and NDVI under drought and heat stress conditions was observed. Genotypes found tolerant against stress has cooler canopy by maintaining the canopy temperature. The findings form this study showed that wheat genotypes higher in SPAD and NDVI also had higher yield, DHTW-60 and C-306 could be used as genetic stock to develop wheat tolerant varieties in breeding programs.

KEYWORDS: Wheat, drought, high temperature, chlorophyll and NDVI.

INTRODUCTION

High-throughput remote sensing phenotyping tools are a rapid and non-destructive methods to plant screening in both field and controlled conditions for high temperature and drought (White *et al.* 2012; Araus and Cairns, 2014) with important consequences for crop improvement. Three recent advanced and non-distractive commonly used traits for high-throughput screening are the normalized difference vegetation index (NDVI), chlorophyll content index (SPAD) and canopy temperature (CT) (Hao *et al.* 2015). NDVI is calculated using wavelengths within the NIR (near infrared) and VIS (visible) regions of the electromagnetic spectrum. NDVI relates to leaf chlorophyll content, leaf nitrogen and ultimately the photosynthetic capacity of the plant (Tattaris *et al.* 2016). CT, which is measured from emitted infra- red radiation, can be used as a tool to indirectly evaluate the transpiration rate, water status and stomatal conductance of a plant (Peñuelas *et al.*1992) while NDVI can estimate relative crop biomass at different growth stages (Babar *et al.* 2006) as well as N deficiency and crop senescence rate (Olivares-villegas *et al.* 2007).

The heat and drought tolerance of wheat can be determined through evaluation of yield performance and physiological traits under individual and combine stress conditions (Reynolds *et al.* 2001; Zhao *et al.* 2007). Chlorophyll content, normalized difference vegetation index (Reynolds *et al.* 1994; Fischer *et al.* 1998) and canopy temperature (Reynolds *et al.* 1994; Amani *et al.* 1996) has also been used as a measure of stress tolerance (Mason *et al.* 2011). Chlorophyll content, normalized difference vegetation index and stay-green trait has also been reported to be associated with heat and drought tolerance in wheat (; Feng *et al.* 2014; Cao *et al.* 2015). The visual observation of stay-green trait has been associated with maintenance of leaf chlorophyll and photosynthetic capacity (Fokar *et al.* 1998). The relationship between NDVI, SPAD and CT under stress conditions and grain yield is well established (Singh *et al.* 2003; Quiring *et al.* 2010). High chlorophyll content, cooler canopy temperature (CT) and maximum normalized difference vegetation index is associated with both drought and heat tolerance (Ramya *et al.* 2015: Munjal and Dhanda, 2016). The aim of present study was to screen the wheat genotypes under independent and combined effects of drought and high temperature on greenness of plant and grain yield under timely, late and very late sown condition.

MATERIALS & METHODS

Raising of crop

Two-year experiment was conducted during winter season of mid-November to April, 2015-16 and 2016-17 with eight selected wheat genotype. In control (irrigated) and drought-stressed experiments (with pre-sowing irrigation only), genotypes were sown at optimum planting date, while for heat-stressed experiment sowing date was delayed. Timely sowing was done on 17 November, 2015 & 13 November, 2016; late sowing on 14 December, 2015 & 16 December, 2016 and very late sowing 13 January, 2016 & 11 January, 2017. The experiment was conducted in the field and laboratory of Wheat and Barley section, Department of Genetics and Plant Breeding, College of Agriculture, CCS HAU, Hisar.

Treatments detail: Drought was created by withholding the irrigation at different stages (40 days after sowing (DAS), 80 DAS, 40+80 DAS (Both at 40 and 80 days after sowing) and for complete drought no irrigation was given throughout the crop season).

Sampling: Five plants of each genotype were randomly tagged for each treatment. Data for NDVI, CCI and CT traits were recorded at anthesis and 21 days after anthesis. Grain yield data were recorded at maturity.

Normalized difference vegetation index (NDVI): NDVI was recorded using optical handheld Instrument Green Seeker^{TM} sensor (Trimble industries, Inc.).

Chlorophyll content index (SPAD): Mean chlorophyll of five tagged plants flag leaves were determined by a SPAD-502 chlorophyll meter (Konica Minolta Sensing, Osaka, Japan).

Canopy temperature (CT): CT was measured during 12.00 and 14.00 h with hand-held infrared thermometer Sixth Sense LT300 IRT and five readings for each plot were averaged to get true representative values.

Yield: Grain yield was recorded after harvesting and thrashing the plot. The thrashed grains were cleaned and yield was recorded in gram.

RESULTS & DISCUSSION

Normalized difference vegetation index (NDVI): NDVI indicate the nitrogen availability, ultimately the photosynthetic capacity of plant. All tolerant and susceptible genotypes showed higher NDVI in timely sown irrigated environments as compare to late and very late drought condition (Fig. 1). Almost of all genotype shows reduction in normalized difference vegetation index at onset of stress whereas, genotype DHTW- 60 and C-306 shows maximum normalized difference vegetation index, followed by HTW-11. Mean normalized difference vegetation index value was 0.84 in timely sown, 0.72 in late sown and 0.69 in very late sown in irrigated condition at anthesis. Combine effects of drought and heat showed mean normalized difference vegetation index value at 21 days after anthesis was 0.49 in irrigated environment (timely sown), 0.39 (late sown) and 0.24 in (very late sown). Our results are documented with Cao *et al.* 2015 and Ramya *et al.* 2016 they found similar result and shows a significant variation between the cultivars for NDVI. NDVI is usually associated with the plant biomass production and yield performance (Govaerts *et al.* 2007).

Chlorophyll content index (SPAD)

Maintaining high leaf chlorophyll content is considered a desirable trait as it indicates a low degree of photo inhibition of the photosynthetic apparatus at high temperatures (Ristic *et al.* 2007; Talebi 2011). Increasing the time and level of stress show reduction in chlorophyll content whereas, genotype DHTW-60, C-306 and HTW-11 was maintaining chlorophyll content at late and very late sown irrigated as well as drought environment. At anthesis (Table 1a) mean chlorophyll content index value was 38.9 in irrigated environment (timely sown), 25.7 (late sown) and 24.0 in (very late sown), combine effects of drought and heat showed mean chlorophyll content index value 24.6 in irrigated environment (timely sown), 17.5 (late sown) and 16.4 plants whereas at 21 days after anthesis (Table 1b) mean chlorophyll content index value was 27.6 in irrigated environment (timely sown), 23.9 (late sown) and 20.4 in (very late sown), combine effects of drought and heat showed mean chlorophyll content index value 20.4 in irrigated environment (timely sown), 17.4 (late sown) and 10.3 (very late sown). Under drought and heat stress condition, chlorophyll content index declined significantly in the present study that was augmented by Fokar *et al.* 1998 and Cao *et al.* 2015.

Canopy temperature (CT)

A cooler CT has been reported to be an important trait associated with grain yield under hot and dry conditions (Kumari *et al.* 2013; Reynolds *et al.* 1994). Canopy temperature at 21 days after anthesis was higher than at anthesis. DHTW-60, C-306 and HTW-11 maintained cooler canopy in all three treatment environments (Irrigated, D40, D80, D40+80 and Drought). Drought stress at anthesis (Table 2 a,b,c) showed mean canopy temperature (^{0}C) 16.0 in irrigated environment (timely sown), 20.0 (late sown) and 26.2 MPa in (very late sown environment), combine effects of drought and heat showed mean canopy temperature (^{0}C) 20.2 in irrigated environment (timely sown), 23.9 (late sown) and 31.0 in whereas at 21 days after anthesis (Table 2 a,b,c) mean canopy temperature (^{0}C) 24.6 in irrigated environment (timely sown), 27.3 (late sown) and 28.8 in (very late sown). Combine effects of drought and heat showed mean canopy temperature (^{0}C) 29.5 in irrigated environment (timely sown), 33.3 (late sown) and 35.0 in very late sown. Similar results have been reported by Fotovat *et al.* (2007) and Hirayama *et al.* (2006). As reported by Mondal *et al.* (2013) CT is a result of complex interaction between environment and several traits, it may not show associations under all environmental conditions.

Yield per plot (g)

Combine effect of drought and high temperature showed greater reduction in yield per plot in both susceptible and tolerant genotype whereas, overall average mean grain yield per plot was higher in DHTW-60 and C-306 followed by HTW-11 (Table 3). Interaction of genotype and treatments resulted in significant decrease of grain yield per plot, however maximum decline was observed at drought 40+80 days after sowing and complete drought in late and very late sown condition.

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TABLE 1 (a): Chlorophyll contents (SPAD) in wheat genotypes at anthesis under timely, late and very late sown conditions

Anthesis	Timely Sown						Late Sown						Very Late Sown					
	Treatments						Treatments						Treatments					
	Control	D80	D40	D40+80	Drought	Mean (G)	Control	D80	D40	D40+80	Drought	Mean (G)	Control	D80	D40	D40+80	Drought	Mean (G)
AKAW 3717	35.2	33.4	33.0	28.9	23.6	30.8	22.3	18.7	20.9	15.4	14.5	18.4	23.8	21.3	20.4	17.3	14.3	19.4
C-306	45.8	43.5	40.9	38.2	32.4	40.2	30.3	28.4	25.0	22.7	23.1	25.9	28.4	26.4	25.5	24.1	23.1	25.5
DHTW-60	51.7	47.0	45.3	42.4	34.4	44.2	32.2	30.7	29.0	28.0	26.9	29.3	29.1	27.6	27.0	25.6	25.0	26.9
HD-2967	35.6	35.1	33.4	28.4	26.9	31.9	24.0	23.7	21.3	15.9	13.3	19.6	23.6	21.5	20.2	19.3	16.9	20.3
HTW-11	42.1	41.4	37.4	35.5	26.5	36.6	26.7	22.6	22.2	20.2	19.8	22.3	26.0	24.0	21.9	18.3	15.0	21.0
KUNDAN	34.2	32.1	26.5	24.1	17.2	26.8	21.3	20.3	19.6	16.5	14.2	18.4	22.5	20.9	16.1	12.0	11.4	16.6
WH-730	29.2	27.8	25.6	23.1	15.1	24.1	21.0	18.4	18.0	15.2	11.8	16.9	17.0	15.9	11.6	10.2	9.7	12.9
WH-1105	37.1	28.6	26.0	24.6	20.4	27.3	24.1	23.8	21.7	17.5	16.3	20.7	22.9	21.6	22.0	20.6	16.2	20.7
Mean (T)	38.9	36.1	33.5	30.6	24.6	32.7	25.2	23.3	22.2	18.9	17.5		24.2	22.4	20.6	18.4	16.4	
CD at 5%	Treatment (T) = 1.51 Genotype (G)= 1.91				TxG=0.14		Treatment (T) = 0.73 Genotype (G)= 0.92				TxG= 2.07		Treatment (T) = 0.64 Genotype (G)= 0.81				TxG= 1.85	

Control- Irrigated, **D40**- Drought at 40 days after sowing (DAS), **D80**- Drought at 80 DAS, **D40+80**- Drought at 40+80 DAS, **Drought**- Complete drought, **T**- Treatments and **G**- Genotype

TABLE 1 (b): Chlorophyll contents (SPAD) in wheat genotypes at 21 days after anthesis under timely, late and very late sown conditions

21 DAA	Timely Sown						Late Sown						Very Late Sown					
	Treatments						Treatments						Treatments					
	Control	D80	D40	D40+80	Drought	Mean (G)	Control	D80	D40	D40+80	Drought	Mean (G)	Control	D80	D40	D40+80	Drought	Mean (G)
AKAW 3717	26.5	23.8	22.0	20.4	18.6	22.3	24.5	23.3	16.9	12.6	11.8	17.8	20.4	19.0	17.6	10.3	9.5	15.4
C-306	31.8	30.6	26.8	25.5	23.3	27.6	27.9	25.4	26.0	21.8	21.2	24.5	26.7	20.4	18.8	14.9	14.0	19.0
DHTW-60	34.3	32.7	29.6	28.1	27.6	30.5	32.7	28.9	29.8	27.4	25.8	28.9	27.3	25.2	22.1	19.1	18.9	22.5
HD-2967	26.6	26.0	24.2	20.7	18.7	23.2	22.5	19.2	21.1	19.9	17.9	20.1	20.2	17.5	14.2	8.7	9.4	14.0
HTW-11	29.7	27.8	26.5	23.9	22.1	26.0	25.9	22.8	24.9	21.7	20.7	23.2	21.8	18.5	17.8	8.2	8.7	15.0
KUNDAN	22.6	19.7	18.6	20.9	19.1	20.2	19.8	16.2	17.0	15.6	14.0	16.5	18.4	14.3	10.4	7.6	8.6	11.9
WH-730	20.7	17.3	16.3	16.6	15.5	17.3	16.7	13.0	14.2	12.7	11.5	13.6	12.4	10.1	8.2	5.1	4.3	8.0
WH-1105	28.7	26.4	22.8	19.1	18.1	23.0	23.5	21.7	20.8	16.0	14.9	19.4	16.2	12.2	9.1	9.1	8.7	11.1
Mean (T)	27.6	25.6	23.3	21.9	20.4	23.8	24.7	22.4	21.8	18.7	17.4		20.4	17.2	14.8	10.4	10.3	
CD at 5%	Treatment (T) = 0.81 Genotype (G)= 1.02				TxG=2.29		Treatment (T) = 0.17 Genotype (G)= 0.22				TxG=0.50		Treatment (T) = 0.11 Genotype (G)= 0.15				TxG=0.38	

Control- Irrigated, **D40**- Drought at 40 days after sowing (DAS), **D80**- Drought at 80 DAS, **D40+80**- Drought at 40+80 DAS, **Drought**- Complete drought, **T**- Treatments and **G**- Genotype

TABLE 2(a): Canopy temperature in wheat genotypes under timely sown conditions

	Anthesis						21 Days after anthesis					
	Treatments						Treatments					
	Control	D80	D40	D40+80	Drought	Mean (G)	Control	D80	D40	D40+80	Drought	Mean (G)
AKAW 3717	16.5	18.7	17.1	18.6	22.3	18.6	26.0	28.2	27.1	29.2	32.1	28.5
C-306	15.6	17.2	16.7	18.0	19.2	17.3	23.7	25.9	24.9	26.7	28.1	25.9
DHTW-60	15.2	17.2	16.6	17.9	18.8	17.1	22.7	25.5	24.6	26.4	27.3	25.3
HD-2967	16.2	18.0	17.0	18.2	20.8	18.0	25.3	27.5	26.3	28.5	30.1	27.5
HTW-11	15.8	17.3	16.8	18.0	19.5	17.5	24.3	26.2	25.3	27.6	28.6	26.4
KUNDAN	16.3	18.5	17.0	18.4	21.2	18.3	25.6	28.0	26.5	28.8	30.9	28.0
WH-730	16.0	17.6	16.8	18.1	19.8	17.7	24.5	26.5	25.7	27.8	29.0	26.7
WH-1105	16.1	17.8	16.9	18.2	20.4	17.9	24.9	27.3	26.1	28.2	29.6	27.2
Mean (T)	16.0	17.8	16.8	18.2	20.2		24.6	26.9	25.8	27.9	29.5	
CD at 5%	Treatment (T) = 0.004 Genotype (G)= 0.005				T×G= 0.012		Treatment (T) = 0.12 Genotype (G)= 0.15				T×G=0.17	

Control- Irrigated, **D40**- Drought at 40 days after sowing (DAS), **D80**- Drought at 80 DAS, **D40+80**- Drought at 40+80 DAS, **Drought**- Complete drought, **T**- Treatments and **G**- Genotype

TABLE 2(b): Canopy temperature in wheat genotypes under late sown conditions

	Anthesis						21 Days after anthesis					
	Treatments						Treatments					
	Control	D80	D40	D40+80	Drought	Mean (G)	Control	D80	D40	D40+80	Drought	Mean (G)
AKAW 3717	21.6	24.0	22.8	24.8	26.7	24.0	29.6	33.9	31.6	35.7	32.8	32.7
C-306	18.9	20.5	19.6	21.1	22.7	20.6	26.0	29.1	27.4	30.0	31.5	28.8
DHTW-60	18.6	19.9	19.1	20.7	21.4	19.9	25.1	28.8	27.1	29.9	31.7	28.5
HD-2967	20.7	23.1	21.7	23.7	24.5	22.7	28.0	32.4	30.1	33.4	33.0	31.4
HTW-11	19.4	21.0	20.2	22.6	23.2	21.3	26.6	29.7	28.4	30.2	33.1	29.6
KUNDAN	21.0	23.8	22.0	24.2	25.3	23.3	28.7	33.0	30.6	34.6	34.2	32.2
WH-730	19.7	21.5	20.8	22.9	23.6	21.7	27.1	30.4	28.6	31.5	34.9	30.5
WH-1105	20.2	22.7	21.4	23.4	24.1	22.4	27.3	31.7	29.2	32.3	35.3	31.2
Mean (T)	20.0	22.1	21.0	22.9	23.9		27.3	31.1	29.1	32.2	33.3	
CD at 5%	Treatment (T)= 0.008 Genotype (G)= 0.010				T×G= 0.023		Treatment (T)= 0.006 Genotype (G)= 0.008				T×G=0.017	

Control- Irrigated, **D40**- Drought at 40 days after sowing (DAS), **D80**- Drought at 80 DAS, **D40+80**- Drought at 40+80 DAS, **Drought**- Complete drought, **T**- Treatments and **G**- Genotype

TABLE 2(c): Canopy temperature in wheat genotypes under very late sown conditions

	Anthesis						21 Days after anthesis					
	Treatments						Treatments					
	Control	D80	D40	D40+80	Drought	Mean (G)	Control	D80	D40	D40+80	Drought	Mean (G)
AKAW 3717	27.1	29.5	28.3	30.4	30.8	29.2	29.9	31.8	30.7	33.2	34.5	32.0
C-306	25.3	28.5	27.3	29.6	30.7	28.3	28.1	31.1	30.1	32.4	33.5	31.0
DHTW-60	23.8	28.4	27.2	29.6	30.6	27.9	26.2	31.1	30.0	32.2	34.0	30.7
HD-2967	26.8	29.3	27.8	30.1	31.0	29.0	29.4	31.5	30.6	33.0	35.0	31.9
HTW-11	26.3	28.7	27.5	29.8	30.9	28.6	28.8	31.3	30.2	32.5	34.6	31.5
KUNDAN	27.0	29.4	28.1	30.2	31.3	29.2	29.7	31.7	30.7	33.1	35.8	32.2
WH-730	26.5	28.9	27.6	29.9	31.3	28.8	29.1	31.3	30.3	32.5	35.8	31.8
WH-1105	26.7	29.2	27.7	30.0	31.7	29.0	29.2	31.4	30.4	32.6	36.8	32.1
Mean (T)	26.2	29.0	27.7	30.0	31.0		28.8	31.4	30.4	32.7	35.0	
CD at 5%	Treatment (T) = 0.009 Genotype (G)= 0.011				T×G= 0.021		Treatment (T) = 0.006 Genotype (G)= 0.008				T×G=0.16	

Control- Irrigated, **D40**- Drought at 40 days after sowing (DAS), **D80**- Drought at 80 DAS, **D40+80**- Drought at 40+80 DAS, **Drought**- Complete drought, **T**- Treatments and **G**- Genotype

A greater decline in grain yield per plot was observed in AKAW-3717 followed by HD-2967, due to interactive effects of combine stresses than DHTW-60 in relation to control in all environment.

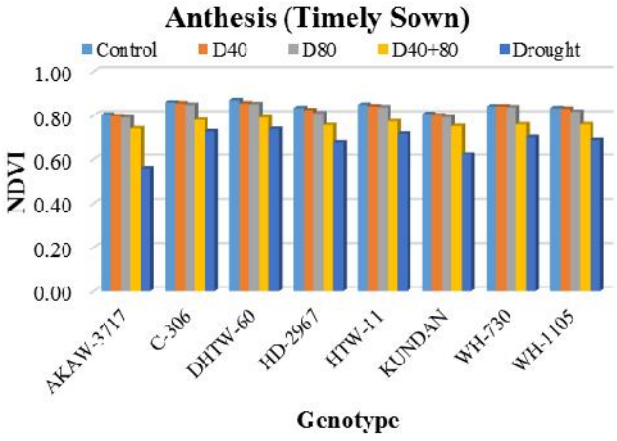
Our results are in agreement with those of Hao *et al.* 2015; Ramya *et al.* 2015; Munjal and Dhanda, 2016 and Tattaris *et al.* 2016 who found normalized difference vegetation index, canopy temperature and chlorophyll content are strongly associated with biomass and yield under normal as well as stress condition. Stress tolerant (drought and heat) varieties were able to maintain higher NDVI. Various studies have shown that the ability of plants to maintain leaf chlorophyll content under high temperatures and drought stress is associated with grain yield and yield components (Ali *et al.* 2010; Yang *et al.* 2002).

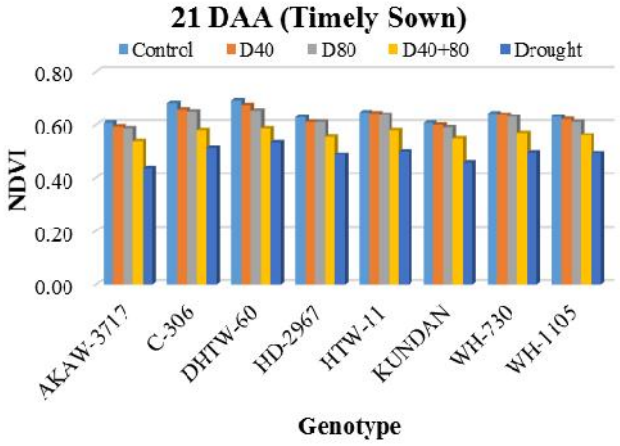
The combined effect of high temperature and drought is more destructive than individual stress alone. DHTW-60, C-306 and HTW-11 genotypes could be used as genetic stock to developed wheat tolerant varieties in breeding programme and NDVI can be used as selection criteria for high yielding wheat genotype under combine stress environments.

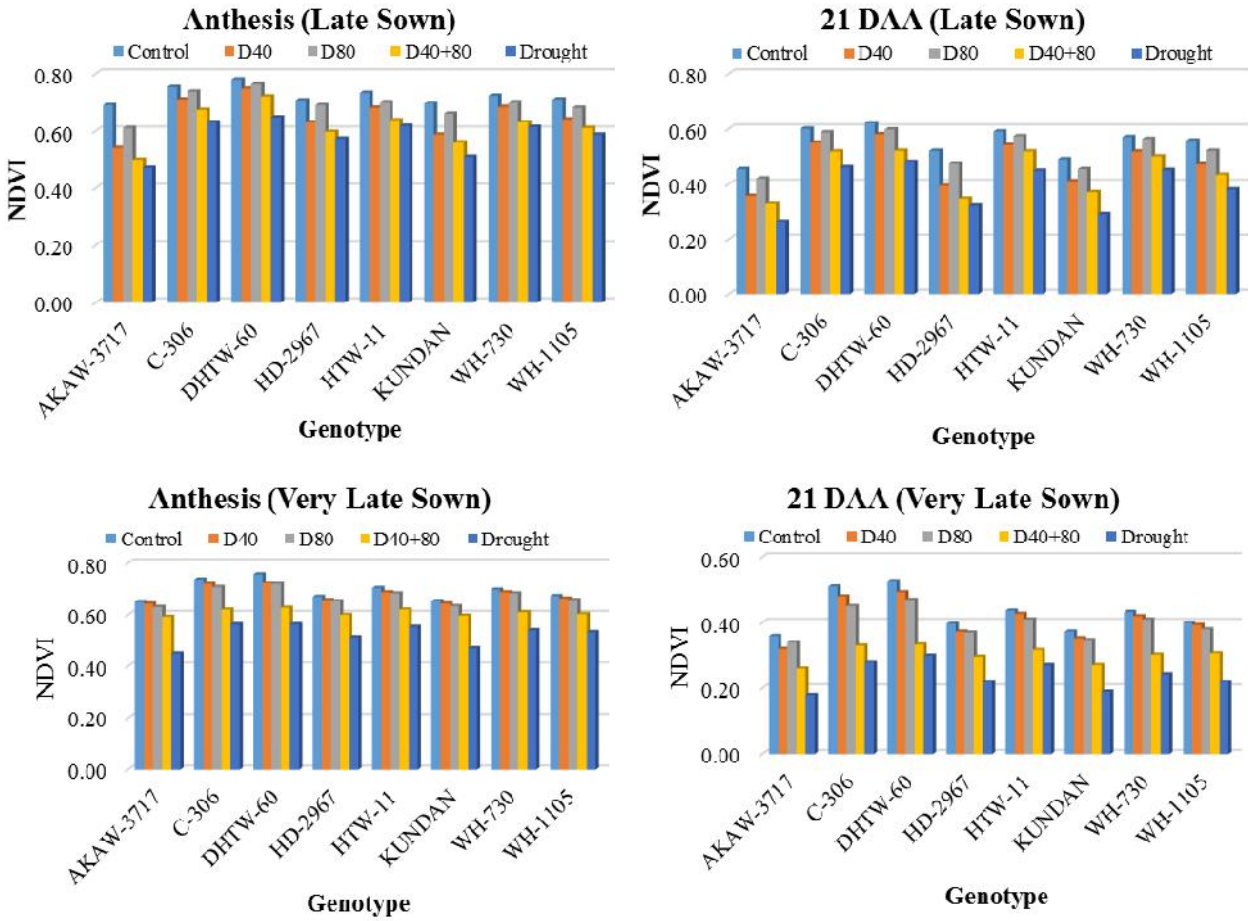
TABLE 3: Grain yield per plot (g) in wheat genotypes under timely, late and very late sown conditions

Genotype/	Timely Sown						Late Sown						Very Late Sown					
	Treatments						Treatments						Treatments					
	Control	D40	D80	D40+80	Drought	Mean (G)	Control	D40	D80	D40+80	Drought	Mean (G)	Control	D40	D80	D40+80	Drought	Mean (G)
AKAW 3717	867.0	644.7	720.0	552.7	261.3	609.1	569.0	426.0	495.3	359.3	185.0	406.9	328.7	180.3	259.7	47.3	34.7	170.1
C-306	1067.7	696.3	816.3	628.7	404.0	722.6	704.0	486.0	553.3	417.0	333.3	498.7	524.7	247.0	318.0	110.3	88.7	257.7
DHTW-60	1237.3	706.0	830.0	638.7	448.0	772.0	796.0	492.0	559.0	425.3	353.7	525.2	534.0	249.7	324.3	118.0	95.3	264.3
HD-2967	912.3	666.3	747.7	565.0	273.3	632.9	586.3	443.7	515.0	389.3	247.3	436.3	347.7	203.7	278.7	71.7	54.7	191.3
HTW-11	945.3	688.0	793.7	601.7	367.3	679.2	652.0	477.7	536.3	411.3	325.0	480.5	494.3	231.3	317.0	99.0	80.0	244.3
KUNDAN	880.7	647.0	736.7	563.7	220.7	609.7	577.7	437.3	498.7	372.7	196.7	416.6	333.7	198.0	273.3	59.3	45.7	182.0
WH-730	926.0	679.3	785.0	586.0	361.3	667.5	632.0	463.0	530.3	407.0	276.7	461.8	453.0	222.3	301.7	91.3	65.0	226.7
WH-1105	924.0	676.7	766.7	573.7	370.3	662.3	601.7	450.3	522.3	398.3	272.7	449.1	358.7	210.0	292.3	80.3	62.0	200.7
Mean (T)	970.0	675.5	774.5	588.8	338.3		639.8	459.5	526.3	397.5	273.8		421.8	217.8	295.6	84.7	65.8	
CD at 5%	Treatment (T) = 10.00				TxG= 28.29		Treatment (T) = 6.90				TxG= 19.52		Treatment (T) = 3.89				TxG= 10.99	
	Genotype (G)= 12.65						Genotype (G)= 8.73						Genotype (G)= 4.92					

Control- Irrigated, **D40**- Drought at 40 days after sowing (DAS), **D80**- Drought at 80 DAS, **D40+80**- Drought at 40+80 DAS, **Drought**- Complete drought, **T**- Treatments and **G**- Genotype







Control- Irrigated, **D40**- Drought at 40 days after sowing (DAS), **D80**- Drought at 80 DAS, **D40+80**- Drought at 40+80 DAS and **Drought**- Complete drought

FIGURE 1: Normalized difference vegetation index (NDVI) in wheat genotypes at anthesis and 21 days after anthesis under timely, late and very late sown conditions

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