

Genotypic variation for normalized difference vegetation index and its relationship with grain yield in wheat under terminal heat stress

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Abstract

The present study was conducted to determine genotypic variations for normalized difference vegetation index (NDVI) at eight different growth stages and its relationship to yield in wheat under terminal heat stress. Thirty six wheat genotypes were evaluated under staggered sowing, timely sowing (15th November, normal), late sowing (15th December, heat stress) and very late sowing (6th January, heat stress) during 2012-13 and 2013-14. The NDVI was recorded at different growth stages viz., flag leaf, booting, heading, anthesis, milk dough, and maturity. The wheat genotypes differed significantly for NDVI at each stage and at each sowing condition. Rate of reduction in NDVI from heading to dough stage was low for genotypes namely, KAUZ/AA//KAUZ, SOKOLL, HW2004, HD2864, HD2967, FLW18, CHIRYA 7 and HD2932. Overall, under heat stress leaf senescence was 7 to 14 per cent faster than normal condition. Genotypes which maintained high NDVI were potential stress tolerant with higher stress tolerance index. Since data recording of NDVI is relatively easier than any other physiological traits, it could serve as indirect selection criteria to measure stress tolerance facilitating selection of the lines from large populations.

Key words: NDVI, wheat, Zadok's scale, genotypic variation, heat stress

Introduction

Terminal heat stress caused by high temperature during grain filling is a major constraint to wheat production in many parts of the world. It is an established fact that high temperature stress can be a significant factor in reducing yield and quality of wheat (Stone Nicolas 1995). Wardlaw (1995) reported that mean temperature greater than 15-18°C following anthesis can result in decrease in grain weight at maturity. According to Ortiz et al. (2008), climate

change is likely to increase the problem of heat stress for wheat production in many parts of Asia. The effects of climatic change are being felt in India, with the most significant impact being experienced in the eastern Indo-Gangetic Plains in the form of shorter winters and the onset of significantly higher temperatures much earlier than normal and a substantial wheat area is heat stressed due to delayed planting (Joshi et al. 2007).

The crop duration hastens when temperatures are elevated between anthesis to grain maturity, grain yield is reduced because of the reduced time to capture resources. Leaf senescence is the progressive loss of green leaf area that occurs during reproductive development of a crop. As plants use the resources to cope with the stress, limited assimilates remain available for reproductive development. Heat stress further triggers the senescence-related metabolic changes in wheat. In addition, inhibition of chlorophyll biosynthesis, degradation of chlorophyll content and breakdown of thylakoid components are also accelerated by heat stress, leading to leaf senescence. Therefore, maintenance of leaf chlorophyll and photosynthetic capacity, called 'stay-green,' is considered an indicator of heat tolerance (Fokar et al. 1998). Because the loss of chlorophyll is associated with less assimilation of current carbon into grains, stay-green genotypes should be better able to maintain grain filling under elevated temperatures.

Different phenological stages differ in their sensitivity to high temperature and this depends on genotype as there are great genetic variations due to geographic locations, adaptability for specific

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environment and selection intensity imposed while breeding for stress tolerance. Traditionally, heat-tolerant genotypes are selected for relatively higher yield under stress conditions. However, selection based on yield *per se* results in a slower response because of over-riding effect of genotype x environmental interactions. This calls for a need for alternative traits that could be used as an indirect selection criterion to improve heat tolerance in wheat. The use of these physiological traits as indirect selection criteria for yield in a breeding program will then depend on their relative importance (genetic correlation with yield), ease and cost of measurement, extent of genetic variation, heritability, genotype x environment interactions, and whether they are associated with adverse pleiotropic effects or genetic linkage (Cossani and Reynolds 2012).

Several studies have proven the fact that the stay-green trait is an indirect selection parameter to improve grain yield in wheat under heat stress (Joshi et al. 2007 and Rosyara et al. 2010). Despite the convenience of measuring the 'stay-green' trait visually, it does not necessarily represent the level of greenness and the photosynthetic capacity (Rosyara et al. 2007). The GreenSeeker® is used to determine the health of green tissues, which could be indicative of grain yield performance. It uses empirical relationships between various vegetation indices and yields (Quarmby et al. 1993; Lobell et al. 2003), providing measurements and data logging of the normalized difference vegetation index (NDVI). NDVI is a mathematical formula derived to form a single spectral-based number, which is more sensitive than just a single wavelength. Sembiring et al. (2000) defined NDVI as the ratio of the difference between near-infrared and the red bands to the sum of the two bands, which is a measure of greenness (chlorophyll content).

Several reports have suggested that NDVI could be used to predict grain yield in cereals (Babar et al. 2006; Teal et al. 2006). NDVI showed significant association with yield under heat stress in two large mapping populations, making it a reliable tool for large scale screening and gene discovery work (Lopes and Reynolds 2012). However, previous studies have reported that NDVI recorded at different growth stages of the wheat crop differed in its relationship with grain yield. Significantly high correlation were reported between grain yield and NDVI recorded at various stages namely booting, heading, grain filling and maturity stages (Freeman et al. 2003; Hazratkulova et al. 2012). Thus, there are contradictory findings on

the relationship between NDVI and grain yield. As abiotic and biotic stresses could greatly influence maturity and performance of wheat, environments could influence the relationship between NDVI and grain yield (Sharma et al. 2008). Genetic variation is most important for improvement of any trait, therefore there is a need to assess genetic variation for NDVI among the genotypes to increase the scope for improvement under heat stress environment. The specific objective of the present study is to examine genetic variation for NDVI at different growth stages and determine the relationship with grain yield and identify genotypes maintaining higher NDVI under terminal heat stress environment.

Materials and methods

The experiment was conducted for two years during *rabi* 2012 and 2013. Material for the present study comprised of 36 bread wheat genotypes, which includes ten lines selected from international core set for abiotic stress developed by International Maize and Wheat Improvement Centre (CIMMYT), Mexico and twenty-six elite Indian lines. The experiments were conducted at experimental farm, Indian Agricultural Research Institute (IARI), New Delhi, India. The latitude of the research farm is 28° 38' 23" N, longitude 77° 09' 27" E and altitude is 228.61 m above mean sea level. The experiment was laid out in a 6x6 simple lattice design with two replications and three dates of sowing with the help of Weintersteiger seed drill. The three different sowing dates *viz.*, 15th November (Timely sowing), 15th December (Late sowing) and 6th January (Very late sowing) in both the year was done to create temperature differences during later growth stages. The gross plot size of timely sown experiment was 1.38 m x 3.0 m with rows at 23 cm apart, whereas in the late and very late sown experiments, the gross plot size was 1.08 x 3.0 m with row-to-row spacing of 18 cm. Standard cultivation practices prescribed for wheat under irrigated conditions were precisely followed.

NDVI was recorded for length of 1 meter in each plot at eight different growth stages starting from flag leaf stages using GreenSeeker® (Trimble, Inc.). NDVI measurements were recorded on dates when all wheat genotypes were near or at Zadoks growth stages (GS) Z36-37 (flag leaf visible), Z-49 (late boot), Z-59 (complete heading), Z-69 (complete anthesis), Z-73 (early milk), Z-77 (late milk) Z-85 (dough) and Z-90 (ripening) (Zadoks et al. 1974). Measurements were made for four to five times in each stage and averaged

to minimize the experimental error. Days to heading and plant heights were recorded in each plot. At maturity, plants in one square meter were harvested from the experimental plots individually to record the grain yield and biological yield since the plot size was not uniform. Data on temperatures were obtained from agro meteorological observatory at IARI experimental farm.

Statistical analysis

Daily mean minimum and maximum data recorded were used to calculate growing degree days (GDD) using 0°C base temperature. GDD was calculated separately for vegetative, anthesis and grain-filling period and for timely, late and very late sown condition for each year. Average GDD per day (GDD/day) was calculated for vegetative, anthesis and grain filling period of the timely and late-seeded crop. Higher GDD/day value was considered as a measure of heat stress. Homogeneity of variance for two experimental years was tested and combined analysis across years was conducted. Statistical analysis was performed with SAS version 9.2 (SAS Institute Inc. 2009) The analysis of variance was done for each stage using SAS mixed model procedure with sowing date and genotypes as fixed, and blocks as random effects. A combined analysis across two years was conducted due to homogeneity of variance. Stress tolerance index (STI) for grain yield was determined by the formula given by Fernandez, (1992), $STI = (X_s * X_n) / Y_n^2$. Where, X_s =phenotypic value under stress of each genotype; X_n =phenotypic value under non stress of each genotype; Y_n = mean phenotypic value of all genotypes under non stress. Rate of reductions in NDVI during dough in late sown and very late sown over timely sown plants were determined to identify the best

genotypes. Correlation coefficients between NDVI and STI were determined for different growth stages at each sowing dates using PROC CORR of SAS.

Results and discussion

Average daily maximum and minimum temperature and relative humidity of the experimental site during crop growth season is presented in Table 1. Temperature during March and April was more than 18°C which was detrimental for grain filling in December (late) and January (very late) sown crop (Wardlaw and Wrigley 1994). The heat stress was created by staggering the sowing dates. There was variation in average growing degree days for three sowing dates determined for vegetative, anthesis and grain filling stages during *rabi* 2012 and 2013 (Table 2). GDD during grain filling stage was 14 (late sown) and 26 (very late sown) percent higher than timely sown during 2012. Likewise, 2013 crop GDD during grain filling was 5 and 28 percent higher in late and very late sown respectively over timely sown crop. This shortened grain filling duration, resulted in lower total GDD for the late sown and very late sown crop. Staggered sowing caused lower GDD both during vegetative and grain-filling period compared to timely sowing which is an indication that the late-sown crop entered post-anthesis period at a lower vigour than the timely-seeded crop. Previous studies have shown that biomass accumulation in wheat is directly related to GDD. The above finding on terminal heat stress and its effect on vegetative and grain-filling period is in agreement with the studies of Sharma (1993).

NDVI which was measured gradually decreases from boot to maturity stage as a result of leaf senescence (Table 3 and Fig. 1). All the genotypes

Table 1. Average maximum and minimum temperatures and relative humidity during the crop season at experimental site for two years

Month	2012-13						2013-14					
	T _{max} °C	T _{min} °C	T _{mean} °C	RH(M) %	RH(E) %	RH mean %	T _{max} °C	T _{min} °C	T _{mean} °C	RH(M) %	RH(E) %	RH mean %
November	27.26	9.93	18.60	89.03	35.03	62.03	26.87	9.93	21.10	90.90	48.27	69.58
December	21.68	7.50	14.59	84.26	49.26	66.76	22.35	7.08	14.72	93.77	55.87	74.82
January	17.99	4.72	11.36	92.03	65.55	78.79	18.60	6.84	12.72	96.65	66.42	81.53
February	22.05	9.63	15.84	91.68	52.14	71.91	21.04	7.51	14.28	95.96	64.68	80.32
March	29.85	13.65	21.75	87.10	35.26	61.18	26.86	12.73	19.79	90.03	48.06	69.05
April	36.06	19.51	27.71	67.20	27.53	47.37	34.77	18.02	26.34	73.43	40.13	56.78

T_{max}=Temperature maximum; T_{min}=Temperature minimum; T_{mean}= Temperature mean; RH(M)= Relative humidity morning; RH(E)=Relative humidity evening

Table 2. Growing degree days (GDD) during vegetative, anthesis, grain filling and total crop seasons based on the temperature at experimental site. Per cent change in GDD due to late sowing and very late sowing. GDD/day for vegetative stages, anthesis and grain filling

Growing degree days (GDD)	Sowing			Per cent change	
	Timely sown	Late sown	Very late sown	Late sown	Very late sown
2012-13					
GDD Vegetative	1065.35	1032.30	1000.60	3	6
GDD Anthesis	305.00	290.85	226.15	5	26
GDD Grain filling	797.00	685.00	588.20	14	26
Total	2190.10	2090.00	1863.00	5	15
GDD/day (Vegetative)	14.02	13.76	15.39	-	-
GDD/day (Anthesis)	15.25	18.18	22.62	-	-
GDD/day (Grain filling)	20.44	24.46	25.57	-	-
2013-14					
GDD Vegetative	1104.60	1003.30	905.50	9	18
GDD Anthesis	277.00	242.50	237.80	12	14
GDD Grain filling	749.30	708.00	540.00	6	28
Total	2124.30	1989.80	1705.50	6	20
GDD/day (Vegetative)	14.50	13.40	14.10	-	-
GDD/day (Anthesis)	13.90	16.20	23.80	-	-
GDD/day (Grain filling)	19.20	25.30	25.70	-	-

showed higher NDVI value at booting (Z49), which started declining in later stages and was low during grain-filling period. Environmental effect was high at Z-36-37, Z-49 and Z-59 due to which coefficient of variation was high. Genotypic variation was low at Z-49 among all the sowing condition, though NDVI measurements were highest during this stage. Hazratkulova (2012) reported narrow range of genetic variation during booting and flowering stages and increased during dough stages. Analysis of variance showed NDVI differed significantly at the eight growth stages at each date of sowing and in each of the year. Thirty six genotypes differed significantly at the eight growth stages at three sowing conditions in each year. Genotypes x growth stages interaction were significant at each sowing condition in each year (Table 4). The significant genotype x growth stage interaction, suggested that relative differences among the genotypes for NDVI changed over growth stages. However, some genotypes maintained higher NDVI in each sowing date. Genotypes EXCALIBUR, PASTOR, MP 4010, HD 2967 and TEPOCA/RABE under timely sown; PASTOR, SOKOLL, HD 2932, EXCALIBUR and CHIRYA 7 in late sown; BAV92/SERI, SOKOLL, CHIRYA 7, PBW 343 and HI 1544 under very late

sown maintained higher NDVI over eight stages. Genotypes such as EXCALIBUR and PASTOR are also superior genotypes for physiological traits which are related to heat stress tolerance and therefore they are widely used as parents to develop heat stress lines in CIMMYT (Reynolds et al. 2001).

Bartlett's test of homogeneity showed that error variances for two years was homogeneous, therefore the data was pooled for the two years to perform correlation and other analysis. Variation for NDVI was significant for main effects of genotypes, date of sowing for each stage; year effect was significant for only three stages (Table 5). Presence of significant variation for NDVI in genotypes x date of sowing interaction shows relative differences among genotypes for NDVI due to staggered sowing. However, genotype x year x sowing date interaction was non-significant for most of the stages. This implies that genotypes perform similarly in both *rabi* 2012 and 2013 under three dates of sowing and therefore it is inferred that NDVI could be stable across the years. The main effect of genotypes and sowing date was significant for grain yield but non-significant for year. Similarly genotype x date of sowing interaction was significant

Table 3. Mean of NDVI, Days for heading, days for maturity, plant height (cms), grain yield (grams/m²) and biological yield for thirty six genotypes

Characters	Timely sown					Late sown					Very late sown				
	Mean	SE	Min	Max	CV	Mean	SE	Min	Max	CV	Mean	SE	Min	Max	CV
	Z36-37	0.79	0.002	0.76	0.82	1.70	0.73	0.006	0.62	0.79	5.16	0.65	0.007	0.53	0.71
Z49	0.84	0.001	0.82	0.86	1.03	0.84	0.002	0.81	0.86	1.35	0.82	0.005	0.76	0.86	3.40
Z59	0.79	0.004	0.73	0.82	2.84	0.77	0.004	0.70	0.82	3.26	0.74	0.005	0.65	0.81	4.37
Z69	0.74	0.004	0.68	0.78	3.40	0.65	0.007	0.56	0.73	6.67	0.60	0.007	0.52	0.67	7.21
Z73	0.69	0.007	0.59	0.77	5.90	0.65	0.005	0.57	0.70	4.17	0.56	0.008	0.47	0.65	8.03
Z77	0.57	0.010	0.43	0.70	11.05	0.38	0.008	0.30	0.50	13.38	0.31	0.007	0.23	0.41	14.33
Z85	0.35	0.006	0.29	0.40	9.54	0.29	0.004	0.24	0.33	8.17	0.22	0.005	0.16	0.27	14.53
Z90	0.29	0.005	0.25	0.34	9.24	0.24	0.003	0.21	0.28	7.67	0.21	0.005	0.15	0.26	14.62
DOH	87.47	0.957	78.00	98.00	6.56	84.87	0.716	78.00	91.00	5.06	69.39	0.453	61.00	74.00	3.91
DOM	136.53	0.483	131.00	145.00	2.12	121.24	0.398	114.00	128.00	1.97	99.07	0.419	87.00	104.00	2.54
PH	94.20	1.861	51.17	111.28	11.85	90.20	1.336	75.63	110.25	8.89	89.19	1.311	75.42	110.92	8.82
GY (grams/m ²)	461.3	16.4	290.9	654.0	10.9	225.2	11.3	131.0	405.1	16.2	92.5	5.2	55.6	193.3	21.7
BY (grams/m ²)	1186.6	42.4	673.5	1814.7	21.4	614.8	25.2	335.9	956.2	24.6	273.2	12.6	140.1	437.1	27.6

Z=Zadoks scale; Z36-37=Flag leaf visible; Z49=Late boot; Z(59)=Complete heading; Z69=Complete anthesis; Z73=Early milk; Z77=Late milk; Z85=Dough and Z90=Ripening; DOH=Days to heading; DOM=Days to maturity; PH=Plant height; GY=Grain yield; BY=Biological yield; H=Harvest index; SE=standard error; Min=Minimum; Max=Maximum; CV=Coefficient of variation

for grain yield. Staggered sowing affected the grain yield due to increase in temperature during grain filling stages in late and very late sowing (Munjal and Dhanda 2004). Days for heading, and maturity period were also reduced due to heat stress for staggered sowings (Table 5).

Rate of reduction in NDVI values from heading to dough stage was 56, 63 and 71 percent for timely sown, late and very late sown crop respectively (Table 6). Therefore under heat stress leaf senescence was 7 to 14 percent faster than normal condition. Genotypes which shows less rate of reduction were KAUZ/AA//KAUZ, SOKOLL, HW2004, HD2864, HD2967, FLW18 and HD2932. The above genotypes also showed superior performance for other stress related physiological traits such as higher stomatal conductance with cooler canopies and Fv/Fm (Ramya 2015). Therefore these genotypes would have been selected under heat stress condition for late sowing regions. Hazratkulova et al. (2012) considered genotypes showing lesser rate of reduction in NDVI from booting to dough stage in heat environment as stress tolerant. The pattern of decline in NDVI in 170 wheat genotypes was studied indicating slower rate of decline in NDVI during grain-filling period with temperatures above 25°C as a measure of heat tolerance (Sharma et al. 2011). Rate of reduction of NDVI from boot to dough stage is due to general phenomenon of senescence associated with maturity. The rapid rate of leaf senescence determined through NDVI, could be due to higher temperature during March, which coincided with early grain filling stages (milk) of the crop. There was 14% increased rate of reduction due to high temperature during March and April leading to early senescence (Joshi et al. 2010). Very late sown crop was affected due to temperature during early stages which is depicted with lesser NDVI value at all the growth stages (Fig. 1).

Stress tolerance index for grain yield was 0.49 and 0.20 under late and very late sown conditions respectively. NDVI at dough stage was significantly correlated with STI (Figs. 2 and 3). Genotypes maintain higher

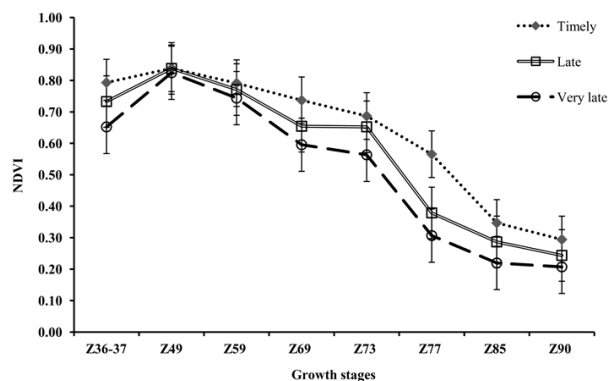


Fig. 1. NDVI measured at different growth stages under timely, late and very late sowing

during January (very late sown). The NDVI was well correlated with stages after flag leaf emergence and after heading under late season crop (Lopes and Reynolds 2012). Significant correlation between NDVI and grain yield from heading to grain filling in wheat has been reported (Sharma et al. 2011; Marti et al. 2007). Higher NDVI values at all the stages among the genotypes during timely sown resulted in non-significant relationship with grain yield. The most appropriate stage for estimating yield was reported to be at mid-grain filling stage (Prasad et al. 2007). Environments could influence the relationship between NDVI and grain yield. NDVI is a measure of chlorophyll

Table 4. Analysis of variance for NDVI at eight growth stages under timely sowing, late sowing and very late sowing during two years

Source	df	2012-13			2013-14		
		Timely sown	Late sown	Very late sown	Timely sown	Late sown	Very late sown
Genotype	35	0.0076**	0.0054**	0.0101**	0.0147**	0.0055**	0.0107**
Growth stages	7	3.3450**	3.7080**	4.0729**	3.1431**	3.7230**	4.0614**
Genotypes X growth stages	245	0.0030**	0.0017**	0.0018**	0.0051**	0.0017**	0.0017**
Replication	1	0.0006	0.0006	0.0004	0.0008	0.0006	0.0004
Error	252	0.0003	0.0003	0.0006	0.0006	0.0003	0.0006

** - Significant at 1% probability level

NDVI are potential to tolerate heat stress. SOKOLL, BABAX, BERKUT and HD2932 had higher STI under late sowing. Similarly genotypes, KAUZ/AA/KAUZ, PBW343, CHIRYA 7, DBW17 and HD2932 show higher STI under very late sowing. Genotypes with higher NDVI at booting and heading stages will maintain higher NDVI at grain filling stages and greater yield potential under severe stress. The temperature was critical for all the growth stages for the crop sown

content in leaves and therefore, maintaining higher NDVI by crop for longer periods during grain-filling would contribute to grain development through photosynthesis. Hence, maintaining higher NDVI under high temperature stress, such as terminal heat during wheat grain filling could be considered a sign of stress tolerance with potential use in germplasm screening.

Multiple recording of NDVI for each growth stage

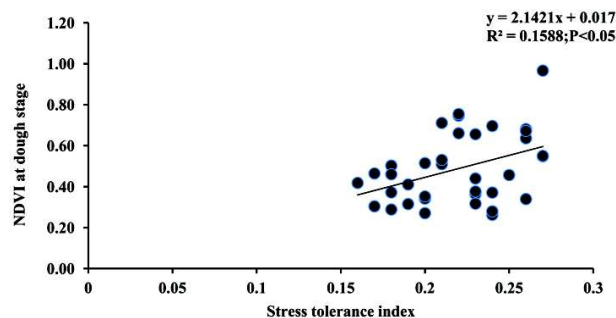


Fig. 2. Regression of stress tolerance index with NDVI at dough stage under late sown condition

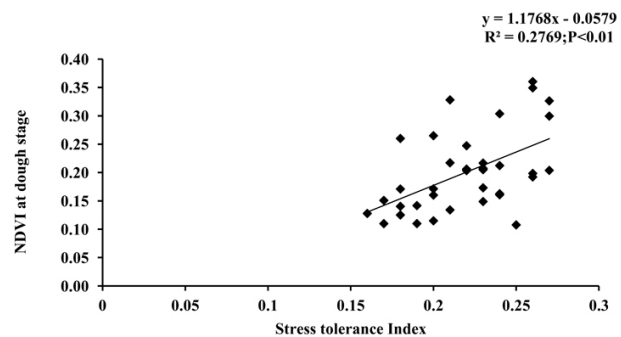


Fig. 3. Regression of stress tolerance index with NDVI at dough stage under very late sown condition

Table 5. Analysis of variance for NDVI at eight growth stages and grain yield under three sowing condition over the years

Source	df	Z36-37	Z49	Z59	Z69	Z73	Z77	Z85	Z90	GY
Genotype (G)	35	0.006166**	0.002099**	0.007677**	0.004008**	0.014008**	0.023653**	0.003319**	0.002555**	58.89**
Date of Sowing (DOS)	2	0.691958**	0.009175**	0.059887**	1.058934**	0.306282**	2.123159**	0.502092**	0.223627**	12288.72**
Year (Y)	1	0.028533ns	0.004552**	0.002446ns	0.000338ns	0.005330**	0.004215**	0.003005ns	0.000862ns	4.80 ns
DOS*Y*R	6	0.001017ns	0.000532ns	0.001412**	0.001209**	0.002442**	0.002340**	0.000396	0.000301ns	6.45 ns
G*S	70	0.003557**	0.000933**	0.005410**	0.004624**	0.003361**	0.013480**	0.005435**	0.004080**	43.62**
G*Y	34	0.000239ns	0.000059ns	0.003268**	0.000257ns	0.000007ns	0.000005ns	0.000004ns	0.000002ns	3.26ns
DOS*Y	2	0.009396**	0.001823**	0.010008**	0.000032ns	0.000145ns	0.000032ns	0.000086ns	0.000022ns	9.66*
G*DOS*Y	68	0.000226ns	0.000058ns	0.003238ns	0.000259ns	0.000005ns	0.000003ns	0.000003ns	0.000002ns	5.19 ns
Error	207	0.000483	0.000261	0.000632	0.000462	0.000077	0.000151	0.000825	0.000622	2.94

*, ** - Significant at 5% and 1% probability level, respectively; ns=non significant; Z=Zadoks scale; DF=Degrees of freedom; GY= Grain yield

Table 6. Reduction in NDVI from heading to dough stage and stress tolerance index (STI) under late and very late sowing conditions

Genotypes	Timely sown		Late sown		Very late sown		STI late sown		STI very late sown	
	54	54	63	59	69	68	0.75	0.20	0.26	0.16
BABAX	54	54	63	59	69	68	0.75	0.20	0.26	0.16
BAV 92/SERI	54	54	63	59	69	68	0.75	0.20	0.26	0.16
BERKUT	57	57	65	65	73	73	0.75	0.25	0.75	0.25
CHIRYA 7	59	59	64	64	65	65	0.55	0.33	0.55	0.33
DBW 14	56	56	64	64	75	75	0.29	0.13	0.29	0.13
DBW 17	60	60	61	61	72	72	0.71	0.33	0.71	0.33
EXCALIBUR	51	51	64	64	71	71	0.51	0.13	0.51	0.13
FLW 18	59	59	61	61	66	66	0.64	0.19	0.64	0.19
GW/273	61	61	64	64	68	68	0.66	0.21	0.66	0.21
GW/322	58	58	66	66	73	73	0.34	0.17	0.34	0.17
GW/366	59	59	60	60	69	69	0.37	0.16	0.37	0.16
HALNA	49	49	60	60	77	77	0.42	0.13	0.42	0.13
HD 2781	49	49	65	65	70	70	0.66	0.21	0.66	0.21
HD 2864	59	59	59	59	72	72	0.53	0.22	0.53	0.22
HD 2932	58	58	67	67	66	66	0.70	0.30	0.70	0.30
HD 2967	53	53	59	59	75	75	0.50	0.26	0.50	0.26
HD 2987	50	50	60	60	73	73	0.51	0.26	0.51	0.26
HI 1544	58	58	64	64	65	65	0.34	0.20	0.34	0.20
HI 1563	50	50	61	61	69	69	0.36	0.21	0.36	0.21
HI 617	61	61	64	64	68	68	0.28	0.21	0.28	0.21
HW 2004	49	49	56	56	67	67	0.46	0.11	0.46	0.11
NW 2036	58	58	65	65	75	75	0.37	0.17	0.37	0.17
KAUZ/AA/KAUZ	55	55	58	58	67	67	0.68	0.36	0.68	0.36
MP 4010	62	62	61	61	75	75	0.31	0.11	0.31	0.11
NI 5439	51	51	61	61	76	76	0.30	0.11	0.30	0.11
PASTOR	52	52	64	64	74	74	0.46	0.15	0.46	0.15
PBW 343	57	57	70	70	68	68	0.67	0.35	0.67	0.35
PBW 550	64	64	68	68	71	71	0.44	0.17	0.44	0.17
RAC 875	58	58	61	61	65	65	0.55	0.20	0.55	0.20
RAJ 3765	52	52	62	62	69	69	0.32	0.15	0.32	0.15
SOKOLL	58	58	60	60	65	65	0.97	0.30	0.97	0.30
TEPOCA/RABE	53	53	65	65	75	75	0.46	0.14	0.46	0.14
WH 147	64	64	66	66	74	74	0.41	0.14	0.41	0.14
WH 730	60	60	67	67	68	68	0.38	0.22	0.38	0.22
WL 711	56	56	65	65	73	73	0.27	0.11	0.27	0.11
WR 544	58	58	61	61	73	73	0.35	0.16	0.35	0.16
General mean	56	56	63	63	71	71	0.49	0.20	0.49	0.20

could eliminate the confounding effect of phenological stages such as days to heading, anthesis and days to maturity. Genotypes, SOKOLL, CHIRYA 7, HI1544 and MP4010 maintained higher NDVI consistently over growth stages and also less percent change over timely sown condition at dough stage. These genotypes are bred for late sown conditions which could harbour genes for stay green and therefore, higher and consistent NDVI. In the present study, we could successfully create heat stress through staggered sowing during December (late sowing) and January (very late sowing) for both the years. Because heat stress was present during grain-filling period, the results provided new information suggesting that NDVI could also serve as an indirect criterion to select for high yield in wheat under terminal heat stress. A proxy trait that can be used as an indirect selection criterion must show high association with the trait of interest and should be suitable to be used in large populations for making measurements rapidly. In addition, genotypic variation should be present among germplasm for such an alternative trait. The present findings showed that NDVI meets these criteria to be considered as an alternative trait to be used in indirect selection. NDVI showed a positive correlation with grain yield and is a rapid method when using a GreenSeeker®. Genotypic variation existed for NDVI at each of the eight growth stages considered in this study and was maximum during grain filling stages. This is particularly important as stress may occur at different growth stages of plants and screening for tolerance to a given stress must be performed in the presence of the stress. Therefore, measuring NDVI, particularly during early milk (Z-73), late milk (Z-77) and dough (Z-85) could increase selection efficiency under heat stress.

Clear understanding of variability patterns is most essential for improvement of the trait. The extent of variability for NDVI at eight growth stages is sufficient to improve the trait under heat stress environment and also the trait per se. Data recording of NDVI is non destructive and high throughput than chlorophyll estimation in laboratory or SPAD. NDVI measurement is relatively easier in field than any other physiological traits. It is emphasized that the physiological trait strategy and physiological trait-based crossing programme will realize cumulative gene action in selected progeny. Such considerable range of variations provided a good opportunity for improvement of NDVI trait which is associated with heat stress tolerance.

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