

Physiological differences at different growth stages of wheat and their effect on yield and yield attributing traits

Chandra Nath Mishra¹, Vinod Tiwari¹, Amit Kumar², Satish Kumar^{1*}, Gyanendra Singh¹ and Gyanendra Pratap Singh¹

¹ICAR-Indian Institute of Wheat and Barley Research, Karnal – 132001 (India)

²ICAR-Research Complex for NEH Region, Umiam, Meghalaya, India

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*Corresponding author

Email: kumarsatish227@gmail.com

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Abstract

India being the second largest wheat producer in the world provides 13% of global wheat supply. Traditional breeding approaches are limited by the low genetic variability, complexity of stress tolerance traits and yield components and the lack of efficient selection techniques under stress conditions. Although the physiological tolerance indices are not directly related to yield but their use might prove increasingly useful. Integration of physiological traits complimented with efficient breeding methodologies will drive the present research to the required wheat yields. A set of twelve wheat lines were used to study the effect of different physiological parameters on the overall yield and its component traits. Progressive increase in NDVI values was noticed during the vegetative phase and a definite decline in values was observed during milk development to ripening stages in the genotypes. In high yielding genotypes a sharp decline of NDVI values was observed from anthesis to milk development stage. This set of genotypes generally showed a slower increase in NDVI values from seedling growth stage to inflorescence emergence stage and faster decline from inflorescence stage to ripening stage. The grain filling occurs in high temperatures and genotypes showing faster utilization of green matter showed higher yield.

Keywords: Wheat, NDVI, CT, Grain Yield, Physiology, Mauchly's sphericity test

1. Introduction

India being the second largest wheat producer in the world provides 13% of global wheat supply (FAO, 2018). Traditional breeding approaches are limited by the low genetic variability, complexity of stress tolerance traits and yield components and the lack of efficient selection techniques under stress conditions. Although the physiological tolerance indices (osmotic adjustment, membrane stability, relative water content etc.) are not directly related to yield but their use might prove increasingly useful. Integration of these physiological traits complimented with efficient breeding methodologies will drive the present research to the required yield heights. Several researches identified physiological traits having

direct correlation with improved genetic yield gains in wheat. Selection techniques based on physiological parameters are now being evaluated for their role as supplementary tool in wheat breeding at CIMMYT (Reynold *et al.*, 2001). Identification of dependable screening methods and efficient selection criteria with physiological input remains a major challenge in traditional breeding programme. Physiological-trait-based breeding is also good strategy and has merit over breeding for yield *per se* because it increases the probability of crosses resulting in additive gene action, although considerable investment in germplasm characterization is prerequisite.

The use of spectral indices as a selection tool in plant breeding could improve genetic gains for different important traits (Babar *et al.*, 2006). Most of these indices have been used for assessment in the last part of crop cycle usually in cereals after anthesis (Marti *et al.*, 2007) and less information is available on the use of Normalized Difference Vegetative Index (NDVI) in relatively early stages. Use of spectral vegetation indices has also been advocated to predict crop status across a broad range of growth stages (Aparicio *et al.*, 2002). Physiological parameters are being effectively used in wheat breeding (Reynold *et al.*, 2007). According to the physiological considerations, survival is the main aim of plant during any stress, whereas from the agricultural point of view yield is the determining drought tolerance trait. In general, change in plant architecture, coleoptile elongation, growth inhibition, scorching of leaves and stems, leaf abscission and senescence are some characteristic symptoms of physiological injuries which have been observed under elevated temperatures and which consequently lead to reduced plant productivity (Tian *et al.*, 2014). Better understanding of the variability available for physiological traits during different phenological stages would help in improving the efficiency of wheat breeding.

Repeated measures are consecutive data obtained over time from the same experimental units such as plants or animals (Littell *et al.*, 1996) and the objectives are to examine and compare response trends over time. The comparisons of treatments can be at specific times, or averaged over time and it also can involve comparisons of times within a treatment (Littell *et al.*, 1998). Repeated ANOVA is applied traditionally for statistical analysis of data including between-subject and within-subject factors and is used safely when sphericity assumption is provided (Eyduran and Akbas, 2010). The sphericity assumption is an assumption about the structure of the covariance matrix in a repeated measures design. It was observed that multivariate methods gave more reliable results than univariate methods (Tabachnick and Fidel, 2001; Gurbuz *et al.*, 2003).

With the above views in consideration the present investigation was carried out to study the time response of the NDVI in a set of wheat genotypes to characterize expressed spatial variability as a function of physiological growth stages.

2. Materials and methods

The present experiment was carried out on nine advanced wheat lines and three check varieties viz., DBW88, WH1105 and HD2967. The parentage details of the tested lines are presented in table 1. All the 12 treatments were grown in Randomized Complete Block Design with three replications during 2016-17 crop season at ICAR-Indian Institute of Wheat and Barley Research Karnal, India. Each plot consisted of 12 rows of 6 meters length spaced 20 cm apart. All the recommended agronomic practices were applied to raise the good crop.

Observations recorded

Agro-morphological traits: Data were recorded on all the treatments for agronomic traits viz., days to heading, plant height, flag leaf width, number of productive tillers per meter, spike length, grain yield and thousand grains weight.

Physiological traits: Data of leaf area index, canopy temperature (CT) and Normalized Difference Vegetative Index (NDVI) were carried out following standard trait dictionary of CIMMYT (Pask *et al.*, 2012). NDVI was measured with the help of hand held Trimble GreenSeeker and was recorded at following eight growth stages:

NDVI 1 (seedling growth stage)

NDVI 2 (tillering stage)

NDVI 3 (stem elongation stage)

NDVI 4 (inflorescence emergence stage)

NDVI 5 (anthesis stage)

NDVI 6 (milk development stage)

NDVI 7 (soft dough stage)

NDVI 8 (ripening stage)

Statistical analysis

The range of dispersion for all the traits was analyzed in SAS 9.3 using Proc MEANS statement. The data of NDVI was observed at 8 intervals for each genotype. Repeated measure analysis was done using GLM procedure of SAS 9.3 using REPEATED statement. This approach basically bypassed the problems of covariance structure rather than addressing them directly. The REPEATED statement enabled users to obtain statistical tests for effects

Table 1: Details of the plant materials used in the study

SN	Entry Name	Pedigree
1	DBW 244	WR1206/F81.513//Milan-1/3/PBW509
2	CA 47th IBWSN 808	BLUEGIL-3/3/NG8675/CBRD//MILAN/4/PSN/BOW//MILAN/5/ KIRITATI/2*TRCH
3	DBW 235	MELON//FILIN/MILAN/3/FILIN/4/TRCH/SRTU//KACHU
4	DBW 224	KACHU/6/YAR/AE.SQUARROSA (783)/4/GOV/AZ//MUS/3/SARA/5/MYNA/ VUL/JUN
5	DBW 166	DANPHE/CHONTE
6	DBW 140	PFAU/MILAN/4/CROC_1/A.SQUARROSA(205)//KAUZ/3/2*ATILLA/3*BCN
7	31st SAWSN 3189	PRL/2*PASTOR*2//FH6-1-7
8	CA 47th IBWSN 938	SWSR22T.B./4/2*BABAX/LR42//BABAX*2/3/KURUKU/5/WBLL1*2/KURUKU
9	LBP 2015-1	HD2733/BH1146
10	DBW 88 (C)	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES
11	WH 1105 (C)	MILAN/S87230//BABAX
12	HD 2967 (C)	ALD/CUC//URES/HD2160M/HD2278

Table 2: Variability and basic statistics observed in the set of genotypes

S.N.	Variable	Range	Mean	Std Dev(SD)	CV (%)	Std Error(SE)
Agronomic traits						
1	Days to heading	89.00 - 118	101.78	7.35	7.22	1.23
2	Plant height (cm)	92.00 - 119.0	103.50	5.88	5.68	0.98
3	Flag leaf width (cm)	1.70 - 3.10	2.09	0.32	15.53	0.05
4	Number of productive tillers per meter	75.00 - 128	95.64	13.51	14.13	2.25
5	Spike length (cm)	7.40 - 12.80	9.83	1.12	11.42	0.19
6	Yield (q/ha)	25.02 - 55.98	47.10	7.19	15.27	1.20
7	TGW (g)	30.98 - 50.20	38.89	5.49	14.12	0.92
Physiological traits						
1	Leaf area index	4.45 - 8.04	5.79	0.83	14.33	0.14
2	CT at heading	12.90 - 18.30	15.48	1.42	9.20	0.24
3	CT at 15DAA	15.60 - 26.10	20.41	2.04	10.01	0.34
4	NDVI1	0.46 - 0.82	0.64	0.09	14.00	0.01
5	NDVI2	0.59 - 0.85	0.74	0.06	8.62	0.01
6	NDVI3	0.67 - 0.89	0.79	0.05	5.95	0.01
7	NDVI4	0.81 - 88	0.85	0.02	1.97	0.00
8	NDVI5	0.80 - 0.86	0.83	0.02	2.01	0.00
9	NDVI6	0.71 - 0.81	0.77	0.03	3.29	0.00
10	NDVI7	0.65 - 0.79	0.73	0.04	4.90	0.01
11	NDVI8	0.55 - 0.77	0.67	0.05	6.74	0.01

involving time trends. Regressions were calculated using REG procedures of SAS 9.3 software (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

Variability for agro-morphological traits studied: Data presented in Table 2. indicates that there was ample amount of variability present in the genotypes tested. The days to heading ranged from 89 to 118 days after sowing with the mean value of 101.8 days. The coefficient of variation for the trait was 7.22%. The average height of the genotypes was 103.5 cm and it varied from 92 to 119 cm (CV 5.68%). The flag leaf width averaged at 2.09 cm and it ranged from 1.73 to 3.10 cm (CV 15.53%). The number of productive tillers per meter ranged from 75 to 128 (CV 14.13%) with mean value of 95.6. Spike length of the genotypes varied from 7.4 to 12.8 cm (CV 11.42%) with mean value of 9.83 cm. The grain yield of genotypes ranged from 25.02 q/ha to 55.98 q/ha (CV 15.27%) and average yield of genotypes was 47.10 q/ha. The 1000 - grains weight of the tested genotypes varied from 30.98 to 50.20 g (CV 14.12 %) with mean value of 38.89 g.

Variability for physiological traits studied: The leaf area index of the genotypes ranged from 4.45 to 8.04 (CV 14.3%) with mean value of 5.79. The Canopy temperature at heading in genotypes varied from 12.9 to 18.30 (CV 9.20%) with mean of 15.48. The CT at 15 DAA had ranged from 15.6 to 26.10 (CV10.01%) with mean of 20.41.

NDVI recorded during eight growth stages i.e. NDVI1 (seedling growth stage), NDVI 2 (tillering), NDVI3 (stem elongation), NDVI4 (Inflorescence emergence), NDVI 5 (anthesis), NDVI 6 (milk development), NDVI 7 (dough development) and NDVI 8 (ripening) showed significant variation. NDVI 1 ranged from 0.46 to 0.82 with average of 0.64, NDVI 2 ranged from 0.59 to 0.85 with average of 0.74, NDVI 3 ranged from 0.67 to 0.89 with average of 0.79, NDVI 4 ranged from 0.81 to 0.88 with average of 0.85, NDVI 5 ranged from 0.80 to 0.86 with average of 0.83, NDVI 6 ranged from 0.71 to 0.81 with average of 0.77, NDVI 7 ranged from 0.65 to 0.79 with average of 0.73, and NDVI 8 ranged from 0.55 to 0.77 with average of 0.67.

Progressive increase in NDVI values was noticed during the vegetative phase in both minimum and maximum values (Fig.1) and definite decline in these values was

observed from NDVI 6 (milk development) to NDVI 8 (ripening stage). The coefficient of variation presented in table 2 shows that maximum variation for NDVI values was observed during seedling growth stage (14.00%), as the genotypes progressed towards tillering stage, the CV reduced to 8.62%, at stem elongation stage it was 5.95, at inflorescence emergence it was minimum (1.97%), there after again the variation started increasing and it was 2.01 at anthesis and 3.29% at milk development stage. At the time of dough development and ripening the coefficient of variation was observed as 4.90% and 6.74%, respectively (Fig 1). The ability of NDVI readings to detect growth stage specific variability provides exciting prospect as past work has shown that early season NDVI readings have been highly correlated with total biomass and yield potential (Raun *et al.*, 2005). It is important to note that peaks for spatial variability were observed at seedling stage indicates the genotypic differences for early vigour and during ripening stage was due to differences among genotypes for decomposition of chlorophyll during grain formation.

The NDVI values observed during the different growth stages in the experiment were subjected to the repeated measure analysis with the objective to understand the change in treatment mean over time and whether there is treatment by time interaction or not.

Between Subject and Within Subject Effect

In repeated measure analysis, two types of factors namely between subject and within subject factor (WSFs) were analyzed as it is quite important to distinguish between these two factors. Between subject factor levels are characterized by independent subjects and provide unrelated observations, whereas within subject factors (WSFs) has levels that represent repeated measurements observed on the same treatment and this repeatedly assessed subjects and give rise to correlated measurements across the levels of any WSFs (Thongbam *et al.*, 2014). Between-subject effects, which is a case of univariate tests of hypotheses are presented in Table 3. The effect of genotypes tested in this experiment were found to be significant ($F= 3.66$) as far as NDVI is concerned. Also within subject effects which include time parameter in addition to genotype and thus incorporates a new dimension of genotype x time interaction, were calculated.

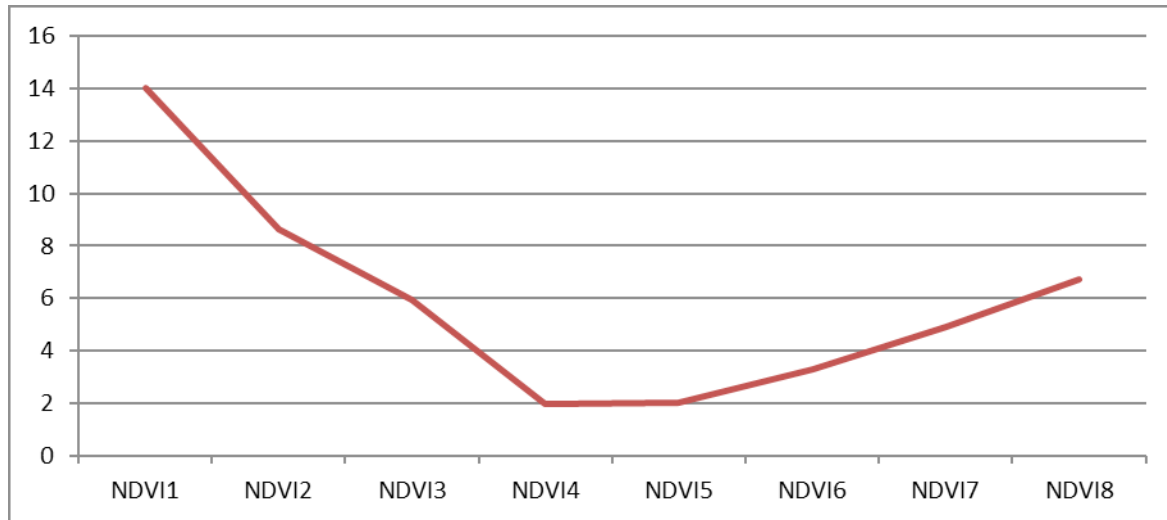


Fig 1: Relationship between the coefficient of variation (CV) for normalized difference vegetative index (NDVI) sensor readings during different growth stages of wheat.

Table 3: Repeated measures analysis of variance for between subject effects (Univariate Tests of Hypotheses)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Genotype	11	0.09719	0.00884	3.66	0.0038
Error	24	0.05789	0.00241		

Both time as well as genotype x time effects were found to be significant revealing that significant difference (table 4) existed in the recordings of NDVI at different stages. This necessitates the selection of superior lines based on NDVI value, which itself is a reflection of high performance of genotypes. Greenhouse- Geisser (G-G) and Huynh-Feldt (H-F) Epsilon adjusted F test approaches are used when validity of sphericity assumption was not provided. Repeated measure ANOVAs being prone to assumption of sphericity that refers to the condition where variances of the differences between all combinations of related groups (levels) are equal, however where these are not equal violation of the sphericity occurs (Table 5). These violations lead to the distortion of variance leading to inflated F ratio. In the present experiment, when these approaches were considered together, time effect was found to be highly significant (<.0001) and interaction effect was significant (<.05).

Mauchly's sphericity test and epsilon adjustment values

When Mauchly's Test of Sphericity is statistically significant ($p < .05$), alternative hypotheses are accepted stating variances of the differences are not equal and sphericity has been violated. This test helps in deciding the

output; when output is univariate, there is more power to reject the null hypothesis otherwise multivariate approach should be followed. In the present experiment probability of Mauchly's test for sphericity assumption was significant ($P < 0.001$). This means that multivariate approach should be chosen instead of univariate approach.

In Multivariate analysis of variance (MANOVA), the main concern is the examination of mean differences when two or more dependent variables are considered simultaneously. However, when the variables are not dependent there is probability of potential interaction which leads to inflation in the error, in such situation MANOVA is most appropriate test. Results of Multivariate analysis of variance are presented in table 6. If the values of Wilks' Lambda is small (close to 0) null hypothesis is rejected. Similarly, if the values of Pillai trace, Hotelling-Lawley Trace, Roy's Greatest Root statistics is large the null hypothesis is rejected. As observed from the table, Wilk's lambda value is close to zero and the value for Pillai trace, Hotelling-Lawley Trace, Roy's Greatest Root statistics is large leading to rejection of null hypothesis on time effect, indicating that NDVI values varied significantly over time. The same is also reflected in terms

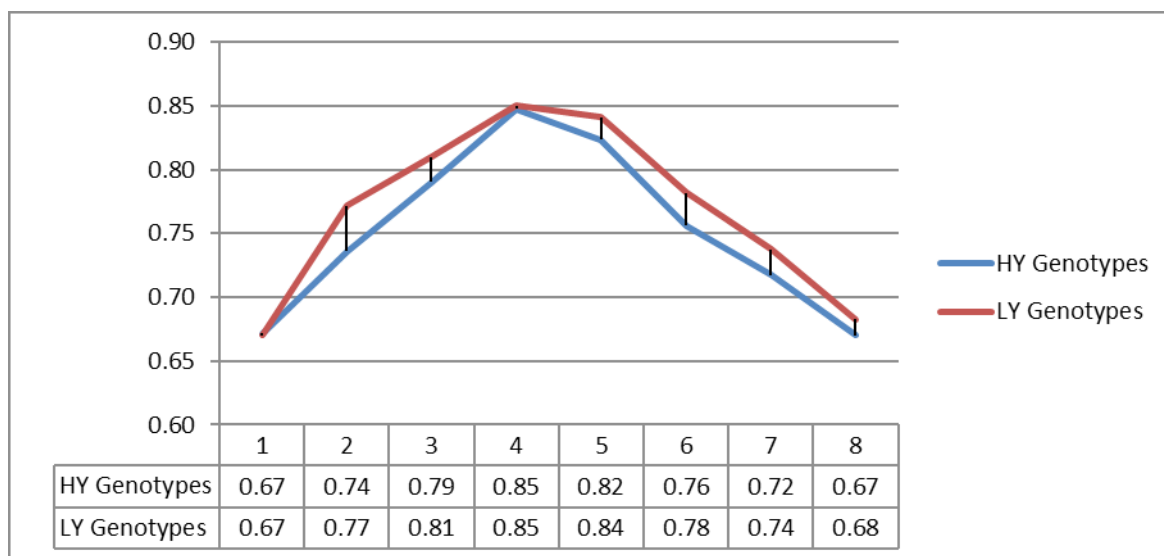


Fig 2: NDVI Pattern in high yielding and low yielding genotypes in different growth stages

Table 4. Repeated measures analysis of variance for within subject effects (Univariate Tests of Hypotheses)

Source	DF	Type III SS	Mean square	F Value	Pr > F	Adj Pr > F	
						G - G	H-F-L
Time	7	1.31356	0.18765	122.81	<.0001	<.0001	<.0001
Time*genotype	77	0.24864	0.00323	2.11	<.0001	0.0153	0.0121
Error(Time)	168	0.25671	0.00153				

Table 5. Sphericity Test results

Mauchly's variables	DF	Criterion	Chi-Square	Pr > ChiSq
Transformed variates	27	0.00028	175.658	<.0001
Orthogonal components	27	0.00028	175.658	<.0001

Table 6. MANOVA Test Criteria and Exact F Statistics for the hypothesis

	Statistic	Value	F Value	Num DF	Den DF	Pr > F
Hypothesis of no Time Effect	Wilks' Lambda	0.00642	398.06	7	18	<.0001
	Pillai's Trace	0.99358	398.06	7	18	<.0001
	Hotelling-Lawley Trace	154.799	398.06	7	18	<.0001
	Roy's Greatest Root	154.799	398.06	7	18	<.0001
Hypothesis of no Time*genotype Effect	Wilks' Lambda	0.00623	2	77	115.31	0.0004
	Pillai's Trace	2.87528	1.52	77	168	0.0131
	Hotelling-Lawley Trace	12.426	2.68	77	53.091	0.0001
	Roy's Greatest Root	7.2933	15.91	11	24	<.0001
Greenhouse-Geisser Epsilon 0.2862, Huynh-Feldt-Lecoutre Epsilon					0.3122	

Table 7. Regression between yield and other attributes for wheat genotypes

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > t
Intercept	1	118.338	83.3249	1.42	0.1726
Leaf area index	1	-0.5767	1.69634	-0.34	0.7378
CT heading	1	-0.0828	0.93759	-0.09	0.9306
CT 15DAA	1	-2.4176	0.80167	-3.02	0.0074
Tiller number	1	0.22341	0.0985	2.27	0.0359
Flag leaf width	1	6.12944	4.70382	1.3	0.209
Height	1	-0.548	0.32358	-1.69	0.1076
DTH	1	0.94612	0.52796	1.79	0.09
Spike length	1	-2.4636	1.34722	-1.83	0.0841
TGW	1	1.07336	0.3915	2.74	0.0134
NDVI1	1	-39.48	25.9127	-1.52	0.145
NDVI2	1	25.4027	34.0923	0.75	0.4658
NDVI3	1	-41.669	39.1211	-1.07	0.3009
NDVI4	1	99.1366	98.9728	1	0.3298
NDVI5	1	-109.94	101.523	-1.08	0.2931
NDVI6	1	-81.672	98.8749	-0.83	0.4196
NDVI7	1	44.4376	74.4953	0.6	0.5583
NDVI8	1	-46.141	72.2419	-0.64	0.5311

of the probability of F value. Thus significant effect of time, and time and genotype interaction was observed in the present experiment. Significant effect of time and genotype interaction shows that the NDVI values varied significantly among different genotypes and also with time. The stage specific variation for NDVI values among different genotypes would provide ample opportunity for selection to be practiced.

In the present study, genotypic variation existed for NDVI at each of the eight growth stages used in this study. Stress may occur any growth stage of plants and therefore screening against a particular stress is understood to be got done in presence of the stress (Hazratkulova *et al.*, 2012).

NDVI Pattern in high yielding and low yielding genotypes in different growth stages:

The mean NDVI values obtained in high yielding genotypes 2nd DBW235, WH1105(C), 2nd DBW224, and CA47th IBWSN 808 and low yielding genotypes DBW140, 31 SAWSN 3189, HD2967 and LBP2015-1 were subjected to graphical analysis presented in Fig 2. The

NDVI values were highest and similar at the inflorescence emergence. Comparable values of NDVI in high yielding and low yielding genotypes at booting and flowering stage was also reported in crops (Thongbam *et al.*, 2014). High NDVI values were noticed in low yielding genotypes at all the rest of stages. In high yielding genotypes sharp decline of NDVI values was observed from anthesis to milk development stage. High-yielding genotypes generally showed a slower increase in NDVI values from seedling growth stage to inflorescence emergence stage and faster decline from inflorescence stage to ripening stage. In the present environment, the grain filling occurs in high temperatures and genotypes showing faster utilization of green matter showed higher yield.

Correlation among various traits

Correlation and regression analysis (Table 7 and 8) on the set of genotypes revealed that there existed some significant correlations between the physiological traits studied and yield as well as days to heading (Morgounov *et al.*, 2014). The NDVI recorded at later stages, particularly NDVI 6,

Table 8. Association among various traits in wheat genotypes

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	-0.22	0.09	0.23	-0.22	0.13	0.12	0.11	-0.02	0.14	0.39*	0.48**	0.24	0.33	0.16	-0.05	-0.13	-0.21
2		0.29	0.16	-0.27	0.26	0.31	-0.04	-0.19	-0.2	0.17	0.06	-0.33*	0.12	0	0.12	0.12	0.22
3			0.46**	0.08	0.23	0.53**	0.38*	-0.58**	-0.27	-0.03	0.13	-0.05	0.17	0.3	0.47**	0.48**	0.46**
4				-0.06	0.06	0.2	0.05	0.03	-0.26	0.14	0.04	-0.12	0.23	-0.11	0	0.08	0.16
5					-0.18	-0.19	0.33*	-0.06	0.07	-0.16	-0.06	0.38*	-0.02	0.23	0.01	0.2	0.14
6						0.58**	0.06	-0.34*	0.01	0.13	-0.11	-0.15	0.17	0.35	0.45	0.44	0.37
7							0.18	-0.56**	-0.53**	0.09	0.01	-0.1	0.17	0.57**	0.75**	0.61**	0.72**
8								-0.37*	0.14	0.15	0.29	0.23	0.4*	0.29	0.05	0.19	0.24
9									0.39	0.03	-0.09	-0.12	-0.23	-0.55**	-0.54**	-0.53**	-0.45**
10										0.38*	0.28	0.24	-0.01	-0.22	-0.51**	-0.45**	-0.46**
11											0.67**	0.38*	0.42**	0.11	-0.4	-0.23	-0.18
12												0.6**	0.23	0.25	-0.26	-0.13	-0.27
13													0.31	0.42**	-0.13	-0.07	-0.23
14														0.33	-0.03	0.02	0.09
15															0.52**	0.51**	0.42**
16																0.71**	0.74**
17																	0.84**

Where, 1-leaf area index, 2-CT heading, 3-CT 15DAA, 4-Filler number, 5-Flag leaf width, 6-Height, 7-DTH, 8-Spike length, 9-Yield (q/ha), 10-TGW, 11-NDVI 1, 12-NDVI 2, 13-NDVI 3, 14-NDVI 4, 15-NDVI 5, 16-NDVI 6, 17-NDVI 7, 18-NDVI 8

NDVI 7 and NDVI 8 showed highly significant correlation with days to heading, overall yield and thousand grains weight trait. In the present environment, the grain filling occurs in high temperatures and genotypes showing faster utilization of green matter showed higher yield. However, this may be a very small set of genotypes to infer any correlation, the results do cohere with the overall outcome of the study. The findings of present investigation have implications for wheat improvement programmes for

developing high-yielding wheat varieties. The optimal stages for measuring NDVI vary depending on the germplasm and environment and little attention is paid by the different breeding programmes for field observations as little visual variation is present between genotypes, but our findings suggest that NDVI measurement at different stages would help in more precise selection of genotypes as there exists significant variation among high and low yielding genotypes at different growth stages.

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