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GENETIC ANALYSIS AND MARKER ASSOCIATION OF PHYSIOLOGICAL TRAITS UNDER RAINFED AND HEAT STRESS CONDITIONS IN SPRING WHEAT (*Triticum aestivum* L.)

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Identifying gene interactions and markers associated with physiological traits, especially at later stages of grain filling, can help develop effective breeding methodology in wheat crop. Six generations (P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2) of four different spring wheat crosses (drought-responsive x drought susceptible) and F_3 generation of a single cross, *i.e.*, MACS6272 x UP2828 were phenotyped and genotyped to decipher gene action and associated markers. Ample variation in canopy temperature depression (CTD – 2.6 – 5.6 °C), chlorophyll content by SPAD (39.6 – 51.3), relative water content (RWC - 51.5 – 75.4 %), grain filling period (GFP - 61.1 – 80.1 days), 100 seed weight (3.7 – 5.5 grams), harvest index (HI - 25.8 – 46.2 %), biological yield (BY – 35.5 – 89.8 grams) and grain yield (GY - 13.4 – 36.5 grams) per plant were observed in six generations. GY positively correlated with CTD, SPAD, 100SW, BY and HI (0.08* - 0.85**). BY had the maximum direct (0.82) and indirect effect via other traits on GY. Significant non-additive epistatic interactions (j & l) and duplicate gene action were found for most traits except GFP and 100SW. Seven different SSR markers associated with CTD, SPAD, NDVI, RWC, 100SW, and explained phenotypic variation (PVE) ranging from 10.1% to 18.4%, with marker *Xcfd35* explaining highest PVE for RWC. The identified candidate genes (in silico) belonged to transmembrane proteins (*Xcfd32*, *Xcfd50*), nucleic acid binding domains (*Xbarc124*, *Xgwm484*) and having enzymatic activity (*Xcfd35*, *Xwmc47*, *Xwmc728*) important for abiotic stress tolerance. Complex inheritance deciphered by six

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generations indicated delaying the selection to later stages of segregation so that useful transgressive segregants can be selected for improving grain yields in wheat.

Key words: Duplicate gene action, epistasis, single-marker analysis, heat stress, wheat

INTRODUCTION

Wheat is an important cereal crop of India and the world, providing nutritional security to millions of people. Global warming and abrupt climatic changes culminating into drought and heat are the major bottlenecks in realizing potential wheat yields. Drought and heat stress affect the wheat crop simultaneously in tropical and sub-tropical environments and central & peninsular India (TIWARI *et al.,* 2014). Simulation models have predicted that there will be a 6% loss in global wheat production with each degree centigrade rise in temperature (ASSENG *et al.,* 2015). Globally, the last 100 years (1906 – 2005) had witnessed increased mean temperatures by 0.74°C and is further predicted to rise by 1.8 to 4.0°C in the next 100 years (IPCC, 2007). In wheat, there is experimental evidence for a 57% reduction in yield by drought, 31% by heat, and 76% by drought and heat together (BALLA *et al.,* 2011). The late sowing of wheat exposes the crop to terminal heat stress and causes a reduction in yield by 25 – 30% (TIWARI *et al.,* 2014). The reproductive stage sensitivity is much higher than the vegetative stage (FAHAD *et al.,* 2017), causing considerable yield losses. Drought stress at the pre-anthesis stage shortens anthesis duration. In contrast, stress post-anthesis reduces grain filling duration mainly by interfering with enzymes involved in starch metabolism (TRICKER *et al.,* 2018; TSHIKUNDE *et al.,* 2019).

Grain yield is a complex quantitative character having a direct or indirect correlation-ship with various morpho-physiological traits. Physiological traits like canopy temperature depression (CTD), chlorophyll content (SPAD), normalized difference vegetation index (NDVI), relative water content (RWC), grain filling duration, seed weight, etc. are reported to have a direct or indirect effect on the grain yield in stress and non-stress conditions and are reported to be reduced in stress conditions (REYNOLDS *et al.,* 2009; TRICKER *et al.,* 2018; TSHIKUNDE *et al.,* 2019). Several radiometric traits like CTD, SPAD, NDVI etc., are gaining popularity among the breeders as the spectral reflectance from the canopy is quickly measurable through handheld devices, which also can be scaled up through aeroplanes or satellite imagery. Importantly, they give an indirect estimation of various agronomic traits, including grain yield in moisture & heat stress environments. The wheat plant in the heat stress environment lowers canopy temperature by stomatal transpiration & several heat-tolerant cultivars are reported to have cooler canopy (ASSENG *et al.,* 2015). SPAD and NDVI give a quick and indirect estimation of leaf photosynthetic health by encompassing variables like ground cover and leaf nitrogen content, thus hinting towards early or late leaf senescence (PINTO *et al.,* 2010; VIJAYALAKSHMI *et al.,* 2010). A cultivar's ability to withstand the post-anthesis loss of chlorophyll content, especially under combined heat & drought stress, results in higher grain yields (TSHIKUNDE *et al.,* 2019). Moreover, canopy temperature and chlorophyll content affect the stomatal conductance, affecting the carbon exchange rate of the leaves and exerts a positive effect on the biomass accumulation, which can be utilized to improve grain yields (REYNOLDS *et al.,* 1994;TSHIKUNDE *et al.,* 2019). Thus, breeding for drought and heat tolerance must involve incorporating

physiological mechanisms by targeting major or multiple physiological traits (DHANDA and SETHI, 2002; TRICKER *et al.,* 2018).

Several researchers have estimated gene effects in the wheat crop for various agronomic traits (ERKUL *et al.,* 2010; SALMI *et al.,* 2019; SAREEN *et al.,* 2018). However, the type of gene action governing the physiological traits is not thoroughly investigated in the past, which can be used for physiological traits-based breeding for improving grain yields in stress environments (AHMAD *et al.,* 2018). Parents and their progeny families are often being used in plant breeding to detect gene effects. The parental lines are crossed in various mating designs (Diallel, line x tester, North Carolina etc.) to create progeny families for partitioning phenotypic variation into several variances such as additive, dominance and epistasis. These variances hint towards the operational intra and inter-locus gene interactions or gene effects (HILL, 2010). Generation mean analysis detects inter-locus epistasis interaction in addition to the intra-locus additive/dominance gene effects as estimated by many other breeding designs, thus enabling a better estimation of the trait in question (KEARSEY and POONI, 2004). Knowledge of gene effects is important, as traits showing dominance gene effects with epistasis need biparental mating or recurrent selection. In contrast, traits with additive gene effects can be improved by pedigree breeding (SAREEN *et al.,* 2018). Identification of markers and underlying genes associated with heat tolerance is important especially in the era of genomic selection. Though many QTLs/genes have been associated with various morpho-physiological traits during heat stress (PINTO *et al.,* 2010; TRICKER *et al.,* 2018) there is further scope to associate markers and genes responsible for heat tolerance, especially in later stages of grain filling. Hence, the present investigation is designed to detect underlying gene effects and associated markers for physiological traits, especially the radiometric traits, which can better equip a breeder to handle these traits in advancing segregating generations to design an effective breeding strategy.

MATERIALS AND METHODS

Planting material

The present investigation was initiated during 2012-13 with the planting of parents and making of F_1 at G. B. Pant University of Agriculture & Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India. Five parental genotypes, three drought responsive, C 306 (REGENT), PBW 175 (HD 2160/WG 1205), MACS 6272 (VORONA/CNO 79//KAUZ/3/MILAN), and two drought unresponsive, UP 2828 (CHOIXM 95/HUW 562) and K 1016 (PBW 373/UP 2338) were used to develop four hybrid combinations *i.e.,* C 306 x K 1016, C 306 x UP 2828, PBW 175 x UP 2828 and MACS 6272 x UP 2828. In 2013-14, each F_1 is backcrossed to either of the parents to create BC_1P_1 (F_1 x P_1) and BC_1P_2 (F_1 x P_2). F_1 was again created in 2013-14 and some of the spikes of F_1, P_1 and P_2 were covered with butter paper to allow self-pollination to harvest seeds of F_2, P_1 and P_2, respectively. During 2014-15, six-generation, i.e., P_1, P_2, F_1, BC_1P_1, BC_1P_2, and F_2 of each of the four crosses were planted in a randomized block design with three replications for each generation population. All six generations were planted in a timely sown, rainfed condition with the recommended package of practices (without artificial irrigation) on 30th October 2014.

Data recording

Data was recorded (no. of individual plants in parentheses) for P_1 (15), P_2 (15), F_1 (30), F_2 (81), BC_1P_1 (24) and BC_1P_2 (24) generations of all three replicates for generation mean analysis. For marker-trait association, F_3 population of the cross MACS 6272 x UP 2828, being highest in F_2 diversity for all studied traits among four populations, was subjected to terminal heat stress by late sown planting conditions in 2015-16, *i.e.*, 15th December 2015, and evaluated for the traits on 72 $F_{2:3}$ individuals. Data were recorded on individual plants for all the studied traits. Three radiometric traits, namely canopy temperature depression (CTD), chlorophyll content (SPAD), and normalized difference vegetation index (NDVI) were observed from 1200 to 1400 hours. CTD was measured as the difference between ambient and canopy temperature (in °C) with the help of a handheld Infra-red thermometer (Spectrum Technologies, Plainfield, IL, USA). Chlorophyll content was estimated using SPAD meter (Model 502, Spectrum Technologies, Plainfield, IL) by averaging the readings for three flag leaves (main shoot and other two tillers at random), and NDVI was measured using a hand device (Trimble® GreenSeeker®).

The other traits observed were grain filling period (GFP) as the difference in the number of days from days to anthesis (Z64) to days to maturity (Z92); 100-grain seed weight (100SW) in grams as a random sample of 100 seeds from an individual plant at harvesting; biological yield per plant (BY) in grams as the weight of total biomass of an individual plant (root + shoot + grain) at harvesting, grain yield per plant (GY) in grams as the total grain weight of an individual plant after harvesting & threshing; harvest index (HI) as the ratio between GY to BY expressed in percentage and relative water content (RWC) at the heading stage (Z55) calculated as:

$$RWC\,(\%) = \frac{(X1 - X3)}{(X2 - X3)} x\ 100$$

Where,

X1 = Fresh weight of 10 cm cut leaf; *X2* = turgid weight (keeping the cut leaf in water for 24 hours) and *X3* = dry weight (keeping the cut leaf at 70 °C in the hot oven for 24 hours); all weights in grams.

Data were recorded at 50% flowering stage (Stage-1) for six generations mean analysis and four different stages, *i.e.*, at 50% flowering stage (Stage-1), 10 days after 50% flowering stage (Stage-2), 20 days after 50% flowering stage (Stage-3) and 30 days after 50% flowering stage (Stage-4) for CTD, SPAD and NDVI in F_3 generation for the marker-trait association.

Biometrical and statistical analysis

Four scales, i.e., $A = 2\overline{BC_1P_1} - \overline{P_1} - \overline{F_1}$; $B = 2\overline{BC_1P_2} - \overline{P_2} - \overline{F_1}$; $C = 4\overline{F_2} - 2\overline{F_1} - \overline{P_1} - \overline{P_2}$; and $D = 2\overline{F_2} - \overline{BC_1P_1} - \overline{BC_1P_2}$ were calculated using the mean of different generations, where $\overline{P_1}$, $\overline{P_2}$, $\overline{F_1}$, $\overline{F_2}$, $\overline{BC_1P_1}$, $\overline{BC_1P_2}$ are the arithmetic means of the first parent, second parent, first filial generation, second filial generation, first backcross generation with 1st parent and first backcross generation with 2nd parent, respectively. The scaling test (scale A, B, C & D) was used to detect the presence of epistasis (MATHER and JINKS, 1982). In the absence of significance of any scale, three-parameter model and in the presence of significance

of one or more scales, six parameter model was used to estimate various gene effects as per MATHER and JINKS (1982)

$$Y = m + a[d] + b[h] + \alpha^2[i] + 2\alpha\beta[j] + \beta^2[l]$$

where, α and β are the coefficients of gene effects, (m) represents mean effect, (d) additive gene effects, (h) dominance gene effects, [i] additive x additive epistatic effects, [j] additive x dominance epistatic effects and [l] dominance x dominance epistatic gene effects (MATHER and JINKS, 1982). [h] and [l] having the same sign was considered complementary epistasis, whereas, opposite signs for [h] and [l] signified duplicate epistasis (KEARSEY and POONI, 2004). The genetic parameters (m, [d], [h], [I], [j], [1]) were tested for significance using a t-test (MATHER and JINKS, 1982). Karl Pearson's simple correlation coefficient was calculated between the traits in six generations of all four crosses. The correlation coefficient between GY and the rest other traits were further partitioned to direct and indirect effects using path analysis (Dewey and Lu, 1959) performed on a pooled data (six generations) of four populations (n=726). All the statistical analysis was carried out using Windostat v 9.1 statistical software.

Genomic DNA isolation and PCR

Genomic DNA was isolated from the parents (MACS6272 and UP2828) and 72 F_3 individuals at 2-3 wheat leaf stage, using the CTAB procedure (Doyle and Doyle, 1990). The DNA concentration was measured using Eppendorf™ UV Biophotometer, and it was diluted to a final DNA concentration of 50 ng/µl for PCR. A total volume of 20 µl was used for a single reaction in a PCR thermocycler (peqSTAR-Peqlab) containing 2µl of 1X Taq buffer (10mM Tris-HCl, 50mM KCl, pH 8.3), 1.5 mM L^{-1} $MgCl_2$, 0.4 µl dNTPs (0.20 mmol L^{-1}), 1 µl (40 ng) of each primer (reverse and forward), 0.2 µl (1U) of Taq-polymerase (GeNei™, Banglore), 14.4 µl deionized water and 1 µl (50 ng) genomic DNA as a template. PCR conditions used were initial denaturing step at 94°C for 5 minutes; 35 cycles of denaturation (94°C for 1 minute), annealing (51 - 61°C, depending upon different primer pair for 1 minute) and extension (72°C for 2 minutes) and, final extension step at 72°C for 7 minutes. Amplicons were separated on 3.0% agarose gel, stained with ethidium bromide, and visualized on the gel documentation system (AlphaImager®, M/s Alpha Innotech, San Leandro, CA).

Marker trait association (MTA)

A set of 250 SSR markers, many of which were randomly distributed over the wheat genome (SOMERS *et al.*, 2004) and few had been associated with biotic and abiotic stresses in the previous experiments, were initially screened for parental polymorphism. Twenty-six polymorphic loci were utilized to genotype 72 F_3 individuals, along with two parents (MACS6272 and UP2828). The SSR markers were scored either dominant or co-dominant. Single marker analysis was used to associate traits with SSR markers using ICiMapping version 4.2 software (MENG *et al.*, 2015). A marker exceeding a threshold of LOD 2.5 was considered significantly associated. The proportion of observed phenotypic variance for a trait explained by a marker is shown as PVE (phenotypic variation explained) in the tabulation.

Candidate genes & their function

Significantly associated markers were projected on the wheat genome to find physical location using BLASTN search function (expect threshold – 10) against the IWGSC Chinese

Spring RefSeq ver. 1.0 on Triticeae Toolbox using viroblast (https://triticeaetoolbox.org/wheat/viroblast/viroblast.php). Candidate genes were identified using JBrowse tool for Chinese spring wheat (http://202.194.139.32/jbrowse-1.12.3-release/) and putative functions of the linked genes were identified using protein databases (Uniprot, Intrerpro and Pfam).

RESULTS

Morpho-physiological traits

In 2014-15, an ample amount of variation in different morpho-physiological traits such as for CTD (2.6 – 5.6 °C), SPAD (39.6 – 51.3), RWC (51.5 – 75.4 %), GFP (61.1 – 80.1 days), 100SW (3.7 – 5.5 grams), BY (35.5 – 89.8 grams), GY (13.4 – 36.5 grams) and HI (25.8 – 46.2 %) was observed among the different generations (Figure 1, Table 1).

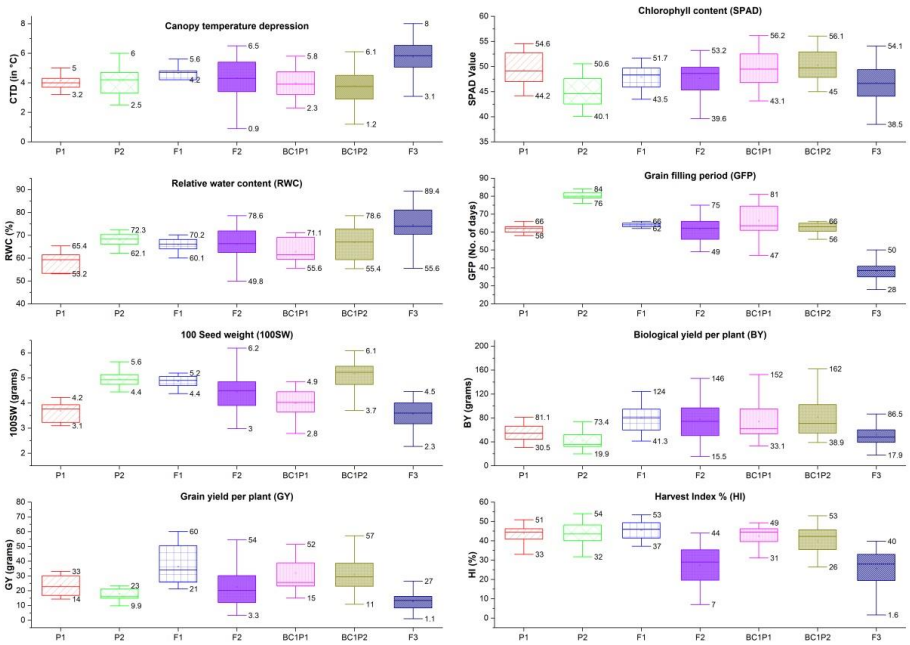

Figure 1. Phenotypic variation for morpho-physiological traits in seven different generations of cross MACS 6272 x UP 2828 in rainfed and heat stress conditions.

Legend: P1 – MACS 6272; P2 – UP 2828; F1 – 1st filial generation of cross (MACS 6272 x UP 2828); F2 – 2nd filial generation of cross; BC1P1 – F1 x MACS 6272; BC1P2 – F1 x UP 2828; F3 – 3rd filial generation of cross.

Table 1. Means and standard errors of six generations for different morpho-physiological trait in wheat

Characters	Generation	C 306 x K 1016		C 306 x UP 2828		PBW 175 x UP 2828		MACS 6272 x UP 2828	
		Mean	S. E	Mean	S. E	Mean	S. E	Mean	S. E
CTD	P_1	2.57	0.17	3.97	0.3	3.91	0.26	4.17	0.17
	P_2	3.17	0.19	4.51	0.23	4.17	0.27	4.13	0.26
	F_1	4.14	0.13	4.36	0.11	4.61	0.28	4.65	0.17
	F_2	4.18	0.13	3.97	0.11	4.05	0.15	4.31	0.14
	BC_1P_1	4.85	0.29	4.26	0.21	5.6	0.23	3.98	0.2
	BC_1P_2	3.55	0.1	3.8	0.19	4.14	0.26	3.77	0.25
SPAD	P_1	39.64	1.25	45.92	1.37	45.01	0.85	49.23	0.86
	P_2	46.07	0.58	43.37	0.79	45.33	0.77	44.66	0.74
	F_1	47.3	0.53	47.12	0.68	48.79	0.47	47.91	0.62
	F_2	47.05	0.56	43.6	0.67	44.68	0.41	47.66	0.41
	BC_1P_1	51.29	1.17	46.34	1.28	46.92	0.73	49.53	0.71
	BC_1P_2	46.34	0.44	45.8	0.83	45.9	0.71	50.24	0.65
RWC	P_1	59.7	2.49	62.56	2.34	74.82	1.8	58.8	1.13
	P_2	59.01	2.97	62.26	2.55	64.39	0.57	67.86	0.8
	F_1	51.51	1.58	67.13	1.9	75.42	0.49	65.88	0.9
	F_2	64.11	1.31	61.3	1.28	72.79	0.69	66.07	0.9
	BC_1P_1	59.69	2.6	68.34	2.07	74.1	0.99	62.89	1.19
	BC_1P_2	65.87	1.81	65.99	2.4	69.82	1.33	67.02	1.47
GFP	P_1	61.87	0.83	62.67	0.33	61.07	0.61	61.33	0.65
	P_2	65.13	0.77	79.6	0.58	78.27	0.44	80.07	0.64
	F_1	66.63	0.53	63.3	0.61	62.8	0.78	64.07	0.37
	F_2	66.44	0.51	65.42	0.49	65.96	0.57	64.22	0.65
	BC_1P_1	63.67	0.73	65.08	0.83	68.63	0.53	67.25	1.36
	BC_1P_2	66.38	0.96	66.46	0.65	65.79	0.69	65.5	0.94
100SW	P_1	4.52	0.12	4.13	0.15	5.10	0.08	3.69	0.10
	P_2	4.39	0.08	5.02	0.12	4.68	0.15	4.95	0.08
	F_1	4.77	0.09	5.45	0.08	5.52	0.09	4.86	0.06
	F_2	4.59	0.07	4.64	0.06	3.98	0.08	4.44	0.07
	BC_1P_1	4.56	0.11	4.56	0.08	4.83	0.07	3.99	0.12
	BC_1P_2	4.56	0.11	5.10	0.13	4.67	0.16	5.16	0.12
BY	P_1	58.51	6.81	52.92	7.62	67.05	4.83	54.03	3.82
	P_2	35.50	4.83	41.78	4.84	46.17	5.25	41.64	3.51
	F_1	60.59	6.19	59.27	6.65	77.04	4.34	79.86	6.46
	F_2	49.78	3.56	67.42	3.65	67.54	3.05	77.52	4.45
	BC_1P_1	89.71	6.25	67.26	5.18	79.41	5.76	74.35	6.39
	BC_1P_2	66.67	6.29	89.76	7.72	64.51	5.14	81.35	7.36

HI	P_1	28.55	3.16	25.81	1.88	42.97	1.09	43.76	1.16
	P_2	38.13	3.46	46.21	2.35	41.11	2.25	43.76	1.54
	F_1	38.66	1.9	36.19	1.92	44.14	2.33	45.4	1.25
	F_2	30.81	1.41	34.12	1.38	28.42	1.27	27.23	1.11
	BC_1P_1	35.10	1.11	30.49	1.77	40.27	1.17	42.4	1.23
	BC_1P_2	40.59	1.64	37.58	2.45	33.56	2.03	39.76	1.83
GY	P_1	16.64	2.49	13.39	2.26	28.65	2.04	23.63	1.77
	P_2	14.97	2.62	19.04	2.11	19.4	2.55	17.98	1.56
	F_1	21.89	1.8	22.34	3.15	34.09	2.82	36.47	3.41
	F_2	15.56	1.33	23.97	1.7	19.58	1.48	22.49	1.79
	BC_1P_1	30.58	1.68	21.09	2.27	31.97	2.49	31.95	3.26
	BC_1P_2	27.29	2.95	34.43	3.61	22.53	2.77	30.99	2.35

CTD – Canopy temperature depression °C; SPAD – Chlorophyll content by SPAD; RWC – Relative water content (%); GFP – Grain filling period (days); 100SW – 100 Seed weight; BY – Biological yield per plant; GY – Grain yield per plant; HI – Harvest Index (%)

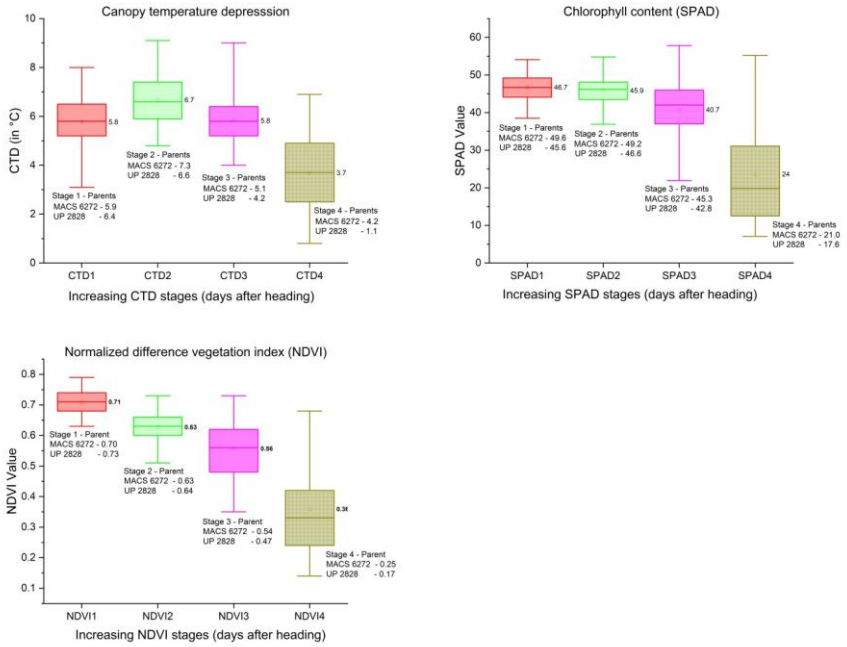

Figure 2. Phenotypic variation for three radiometric traits (CTD, SPAD and NDVI) in four different stages post heading in F_3 generation of cross MACS 6272 x UP 2828 in heat stress conditions.

Legend: CTD1 – 4 (CTD1 – CTD at 50% heading stage; CTD2 - CTD at 10 days after 50% heading stage, CTD3 - CTD at 20 days after 50% heading stage; CTD4 - CTD at 30 days after 50% heading stage); SPAD1 – 4 (SPAD1 – SPAD at 50% heading stage; SPAD2 - SPAD at 10 days after 50% heading stage, SPAD3 - SPAD at 20 days after 50% heading stage; SPAD4 - SPAD at 30 days after 50% heading stage). NDVI1 – 4 (NDVI1 – NDVI at 50% heading stage; NDVI2 - NDVI at 10 days after 50% heading stage, NDVI3 - NDVI at 20 days after 50% heading stage; NDVI4 - NDVI at 30 days after 50% heading stage).

Scaling test and generation mean analysis

Table 2. Scaling test results for four different wheat crosses in rainfed conditions

Trait	Cross	A	B	C	D
CTD	C306 x K1016	2.99* ± 0.62	-0.22 ± 0.31	2.70* ± 0.64	-0.04 ± 0.40
	C306 x UP2828	0.18 ± 0.52	-1.28* ± 0.46	-1.34* ± 0.63	-0.12 ± 0.36
	PBW175 x UP2828	2.68* ± 0.60	-0.51 ± 0.65	-1.11 ± 0.89	-1.64* ± 0.46
	MACS6272 x UP2828	-0.86 ± 0.47	-1.24* ± 0.59	-0.35 ± 0.73	0.88* ± 0.43
SPAD	C306 x K1016	15.63* ± 2.70	-0.69 ± 1.18	7.88* ± 2.82	-3.53* ± 1.67
	C306 x UP2828	-0.35 ± 2.98	1.12 ± 1.96	-9.12* ± 3.40	-4.94* ± 2.03
	PBW175 x UP2828	0.03 ± 1.75	-2.33 ± 1.69	-9.21* ± 2.21	-3.46* ± 1.31
	MACS6272 x UP2828	1.92 ± 1.78	7.91* ± 1.63	0.9 ± 2.34	-4.47* ± 1.26
RWC	C306 x K1016	8.18 ± 5.98	21.21* ± 4.94	34.70* ± 7.24	2.65 ± 4.11
	C306 x UP2828	6.99 ± 5.13	2.59 ± 5.76	-13.88 ± 7.24	-11.73* ± 4.07
	PBW175 x UP2828	-2.02 ± 2.72	-0.17 ± 2.76	1.13 ± 3.48	1.66 ± 2.16
	MACS6272 x UP2828	1.1 ± 2.78	0.29 ± 3.17	5.85 ± 4.25	2.23 ± 2.61
GFP	C306 x K1016	-1.17 ± 1.76	0.98 ± 2.13	5.51* ± 2.55	2.85 ± 1.57
	C306 x UP2828	4.20* ± 1.80	-9.98* ± 1.54	-7.19* ± 2.40	-0.7 ± 1.43
	PBW175 x UP2828	13.38* ± 1.45	-9.48* ± 1.64	-1.08 ± 2.86	-2.49 ± 1.43
	MACS6272 x UP2828	9.10* ± 2.82	-13.13* ± 2.03	-12.64* ± 2.84	-4.31* ± 2.10
100SW	C306 x K1016	-0.18 ± 0.26	-0.03 ± 0.24	-0.10 ± 0.34	0.06 ± 0.2
	C306 x UP2828	-0.47* ± 0.22	-0.27 ± 0.29	-1.49* ± 0.33	-0.38* ± 0.19
	PBW175 x UP2828	-0.96* ± 0.18	-0.87* ± 0.36	-4.89* ± 0.38	-1.53* ± 0.23
	MACS6272 x UP2828	-0.58* ± 0.26	0.51* ± 0.25	-0.62 ± 0.34	-0.27 ± 0.22
BY	C306 x K1016	60.31* ± 15.52	37.25* ± 14.82	-16.08 ± 20.63	-56.82* ± 11.36
	C306 x UP2828	22.32 ± 14.47	78.47* ± 17.49	56.43* ± 21.71	-22.18 ± 11.82
	PBW175 x UP2828	14.74 ± 13.22	5.81 ± 12.33	2.86 ± 16.57	-8.84 ± 9.83
	MACS6272 x UP2828	14.81 ± 14.82	41.18* ± 16.45	54.69* ± 22.59	-0.65 ± 13.19
GY	C306 x K1016	22.63* ± 4.55	17.73* ± 6.70	-13.17 ± 7.38	-26.76* ± 4.31
	C306 x UP2828	6.45 ± 5.96	27.47* ± 8.15	18.78 ± 9.76	-7.57 ± 5.45
	PBW175 x UP2828	1.19 ± 6.07	-8.42 ± 6.72	-37.91* ± 8.79	-15.34* ± 4.76
	MACS6272 x UP2828	3.8 ± 7.56	7.53 ± 6.02	-24.60* ± 10.17	-17.97* ± 5.38
HI	C306 x K1016	2.99 ± 4.30	4.39 ± 5.13	-20.77* ± 8.24	-14.08* ± 3.43
	C306 x UP2828	-1.03 ± 4.44	-7.23 ± 5.77	-7.92 ± 7.38	0.17 ± 4.10
	PBW175 x UP2828	-6.57 ± 3.48	-18.13* ± 5.20	-58.66* ± 7.33	-16.98* ± 3.45
	MACS6272 x UP2828	-4.36 ± 2.99	-9.64* ± 4.16	-69.41* ± 5.45	-27.70* ± 3.13

"*" and "**, Significant at $p<0.05$ and $p<0.01$ level of significance, respectively. CTD – Canopy temperature depression °C; SPAD – Chlorophyll content by SPAD; RWC – Relative water content (%); GFP – Grain filling period (days); 100SW – 100 Seed weight; BY – Biological yield per plant; GY – Grain yield per plant; HI – Harvest Index (%)

For the eight studied traits, one or another scale was significant in four selected crosses. However, in two crosses for RWC and one cross each for 100SW, BY, and HI, none of the scales were significant. This indicated absence of epistasis and inheritance to be simply explained by the additive-dominance model. In the rest of the crosses for all traits, the significance of either one or more scales showed the presence of digenic interactions (Table 2).

Dominance gene effect [h] was positive and significant for SPAD, 100SW, BY, GY and HI in most of the crosses. Negative and significant dominance x dominance [l] epistatic interaction was found for almost all the studied traits in the majority of the crosses. Thus, the opposite direction of gene effects (h and l) for most traits, especially SPAD, 100SW, BY, GY, and HI, indicates their complex inheritance and probable segregation generation after generation until they become homozygous (Table 3).

Table 3. Generation mean analysis results for four different wheat crosses in rainfed condition

Trait	Cross	m+se	d+se	h+se	i+se	j+se	l+se	Epistasis
CTD	[b]C306 x K1016	4.18* ± 0.13	1.31* ± 0.31	1.35 ± 0.82	0.07 ± 0.80	1.61* ± 0.33	-2.85* ± 1.38	–
	[b]C306 x UP2828	3.97* ± 0.11	0.46 ± 0.28	0.37 ± 0.76	0.25 ± 0.73	0.73* ± 0.34	0.85 ± 1.30	–
	[b]PBW175 x UP2828	4.05* ± 0.15	1.46* ± 0.35	3.86* ± 0.98	3.28* ± 0.92	1.59* ± 0.40	-5.46* ± 1.66	Duplicate
	[b]MACS6272 x UP2828	4.31* ± 0.14	0.21 ± 0.32	-1.25 ± 0.89	-1.75* ± 0.86	0.19 ± 0.36	3.85* ± 1.48	–
SPAD	[b]C306 x K1016	47.05* ± 0.56	4.95* ± 1.35	11.50* ± 3.46	7.06* ± 3.25	8.16* ± 1.43	-21.99* ± 5.74	Duplicate
	[b]C306 x UP2828	43.60* ± 0.67	0.54 ± 1.53	12.36* ± 4.2	9.89* ± 4.07	-0.73 ± 1.72	-10.65 ± 6.99	–
	[b]PBW175 x UP2828	44.60* ± 0.41	1.02 ± 1.02	10.54* ± 2.72	6.92* ± 2.62	1.18 ± 1.17	-4.62 ± 4.65	–
	[b]MACS6272 x UP2828	47.66* ± 0.40	-0.71 ± 0.97	9.90* ± 2.66	8.93* ± 2.52	-3.00* ± 1.12	-18.76* ± 4.52	Duplicate
RWC	[b]C306 x K1016	64.11* ± 1.21	-6.18 ± 3.17	-13.15 ± 8.59	-5.31 ± 8.33	-6.52 ± 3.71	-24.08 ± 14.59	
	[b]C306 x UP2828	61.30* ± 1.27	2.35 ± 3.17	28.18* ± 8.54	23.47* ± 8.14	2.20 ± 3.61	-33.05* ± 14.61	Duplicate
	[a]PBW175 x UP2828	72.92* ± 4.41	5.21* ± 0.04	-3.02 ± 11.72				Absent
	[a]MACS6272 x UP2828	67.78* ± 5.26	-4.53* ± 0.69	-4.95 ± 13.62				Absent
GFP	[b]C306 x K1016	66.44* ± 0.51	-2.71* ± 1.20	-2.56 ± 3.24	-5.69 ± 3.14	-1.08 ± 1.33	5.88 ± 5.44	–
	[b]C306 x UP2828	65.42* ± 0.49	-1.38 ± 1.05	-6.43* ± 2.95	1.4 ± 2.87	7.09* ± 1.10	4.38 ± 4.84	–
	[b]PBW175 x UP2828	65.96* ± 0.57	2.83* ± 0.87	-1.89 ± 2.99	4.98 ± 2.86	11.43* ± 0.95	-8.88* ± 4.50	–
	[b]MACS6272 x UP2828	64.22* ± 0.65	1.75 ± 1.66	1.98 ± 4.24	8.61* ± 4.20	11.12* ± 1.72	-4.58 ± 7.21	–
100SW	[a]C306 x K1016	4.56* ± 0.41	0.06 ± 0.07	-0.12 ± 1.69				Absent
	[b]C306 x UP2828	4.64* ± 0.06	-0.54* ± 0.15	1.63* ± 0.29	0.75* ± 0.27	-0.10 ± 0.17	-0.01 ± 0.67	–
	[b]PBW175 x UP2828	3.98* ± 0.07	0.16 ± 0.17	3.69* ± 0.47	3.07* ± 0.45	-0.05 ± 0.19	-1.24 ± 0.79	–
	[b]MACS6272 x UP2828	4.44* ± 0.07	-1.18* ± 0.16	1.08* ± 0.44	0.54 ± 0.44	-0.54* ± 0.17	-0.47 ± 0.73	–
BY	[b]C306 x K1016	49.78* ± 3.56	23.04* ± 8.86	127.23* ± 23.92	113.64* ± 22.73	11.53 ± 9.80	-211.20* ± 41.02	Duplicate
	[b]C306 x UP2828	67.42* ± 3.65	-22.50* ± 9.29	56.28* ± 24.96	44.35 ± 23.64	-28.07* ± 10.33	-145.14* ± 43.05	Duplicate

	[a]PBW175 x UP2828	38.93 ± 19.99	10.44* ± 3.57	76.34 ± 53.58				Absent
	[b]MACS6272 x UP2828	77.52* ± 4.45	-6.99 ± 9.75	33.33 ± 27.29	1.3 ± 26.39	-13.19 ± 10.08	-57.3 ± 45.05	–
GY	[b]C306 x K1016	15.56* ± 1.33	3.29 ± 3.39	59.62* ± 8.99	53.53* ± 8.62	2.45 ± 3.84	-93.88* ± 15.44	Duplicate
	[b]C306 x UP2828	23.97* ± 1.70	-13.34* ± 4.26	21.26 ± 11.44	15.13 ± 10.89	-10.51* ± 4.53	-49.04* ± 19.63	–
	[b]PBW175 x UP2828	19.58* ± 1.48	9.43* ± 3.73	40.76* ± 10.05	30.69* ± 9.51	4.81 ± 4.07	-23.46 ± 17.3	–
	[b]MACS6272 x UP2828	22.49* ± 1.79	0.96 ± 4.02	51.59* ± 11.35	35.93* ± 10.77	-1.86 ± 4.19	-47.26* ± 19.02	Duplicate
HI	[b]C306 x K1016	30.81* ± 1.40	-5.48* ± 1.07	33.47* ± 7.50	28.15* ± 6.87	-0.7 ± 3.06	-35.53* ± 11.41	Duplicate
	[a]C306 x UP2828	36.35* ± 8.34	-10.20* ± 1.50	-8.77 ± 21.81				Absent
	[b]PBW175 x UP2828	28.42* ± 1.27	6.71* ± 2.35	36.06* ± 7.40	33.96* ± 6.91	5.78* ± 2.66	-9.26 ± 11.9	–
	[b]MACS6272 x UP2828	27.23* ± 1.11	2.64 ± 2.20	57.05* ± 6.45	55.40* ± 6.25	2.64 ± 2.41	-41.40* ± 10.36	Duplicate

m – mean effect, d – additive effect, h – dominance effect, i – additive x additive; j – additive x dominance; l – dominance x dominance epistatic gene interaction

"*" and "**, Significant at $p<0.05$ and $p<0.01$ level of significance, respectively. CTD – Canopy temperature depression °C; SPAD – Chlorophyll content by SPAD; RWC - Relative water content (%); GFP – Grain filling period (days); 100SW – 100 Seed weight; BY – Biological yield per plant; GY – Grain yield per plant; HI – Harvest Index (%).[a] Results for three-parameter model (in absence of epistasis); [b] Results for six-parameter model (in presence of epistasis)

Correlation and Path analysis

Table 4. Direct and indirect effects of various physiological traits on grain yield per plant

Traits	CTD	SPAD	RWC	GFP	100SW	BY	HI	r
CTD	**-0.0092**	-0.0009	-0.0007	-0.0004	0.0000	0.0659[a]	0.0273	0.08*
SPAD	-0.0014	**-0.0055**	-0.0015	0.0002	0.0025	0.1756[a]	0.0468	0.22**
RWC	0.0004	-0.0005	**-0.0158**	-0.0006	-0.0018	0.0770[a]	-0.0469	0.012[NS]
GFP	-0.0003	0.0001	-0.0007	**-0.0130**	0.0030	-0.1184[a]	0.1007	-0.029[NS]
TW	0.0000	-0.0005	0.0009	-0.0013	**0.0309**	0.0593	0.1139[a]	0.20**
BY	-0.0007	-0.0012	-0.0015	0.0019	0.0023	**0.8152**	0.0350[a]	0.85**
HI	-0.0006	-0.0006	0.0016	-0.0029	0.0077	0.0622[a]	**0.4587**	0.53**

"*" and "**, Significant at 5% and 1% level of probability; NS – Non-Significant. CTD – Canopy temperature depression °C; SPAD – Chlorophyll content by SPAD; RWC – Relative water content (%); GFP – Grain filling period (days); 100SW – 100 Seed weight; BY – Biological yield per plant; HI – Harvest Index (%)

r – Pearson correlation coefficient between the trait and grain yield per plant. Sum of all direct and indirect effects equals to correlation coefficient ('r' value) between a trait and grain yield per plant.

Bold values: Direct effects.

[a] Highest indirect effect for a particular trait

CTD and SPAD were positively correlated in two crosses. BY was positively correlated to CTD, SPAD and 100SW in two, three and two crosses, respectively (Figure 3). GY is an overall result of the interaction of studied traits. It was positively correlated with CTD, SPAD, and 100SW for two, three, and two crosses, respectively. Moreover, it was significantly and positively correlated with BY and HI for all the studied crosses (Figure 3, Table 4). Path analysis showed that the highest direct effect on GY was exerted by BY (0.82), followed by HI (0.46) and TW (0.03). The radiometric traits (CTD, SPAD) influenced GY majorly via the indirect path of BY. In fact, all traits except TW and BY affected the GY indirectly by influencing the BY. TW and BY exerted an indirect effect (positively) on GY via HI (Table 4).

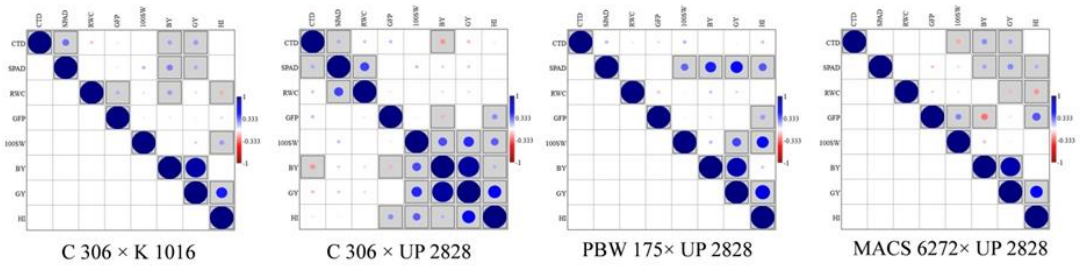

Figure 3. Correlation between morpho-physiological traits in four different wheat crosses in rainfed conditions

Legend: Note: Significant correlation ($p<0.05$) for Karl Pearson correlation coefficient are shown in rectangular boxes

Marker trait association, Candidate genes and their functions

In the present investigation, the F_3 population was planted in late conditions, *i.e.*, 15th December 2015, to associate markers with terminal heat stress post-flowering. Considerable variation among the parents and a good amount of transgressive segregation was observed in the F_3 generation (Figure 1 & 2). Altogether seven different SSR markers on five different chromosomes are associated with the studied traits. Significantly associated markers for all three radiometric traits at various stages, i.e., CTD (1st), SPAD (4th) and NDVI (2nd and 3rd), were found with PVE ranging from 10.1 – 15.9 %. Marker *Xcfd35* located on chromosome 3D, explained the highest phenotypic variation of 18.4% for RWC (Table 5). Markers *Xgwm484* and *Xcfd50* were associated with the maximum number of traits, i.e., commonly to NDVI (stage 2 and 3) and SPAD-4 and 100SW, respectively. The candidate genes and predicted proteins for the significant SSR markers were associated with transmembrane proteins (*Xcfd32*, *Xcfd50*), nucleic acid binding domains (*Xbarc124*, *Xgwm484*), and having enzymatic activity, i.e., oxidoreductases (*Xcfd35*, *Xwmc47*) and cysteine protease-like proteins (*Xwmc728*), all-important to provide abiotic/biotic stress tolerance to a plant (Table 5).

Table 5. Marker trait association, candidate genes and their putative functions detected in F_3 population of wheat cross MACS6272 x UP2828 (Markers with LOD score ≥ 2.50 are shown in the table)

Sr. No.	Marker	Chromosome /arm	Position (Mb)	Trait associated	LOD score	Additive effect	PVE (%)	Candidate gene ID	Putative function	Previous association with marker	Reference
1.	*Xwmc728*	1BL	685.2	100SW	4.9	0.15	16.3	TraesCS1B01G476300.1	Cysteine protease-like protein	Grain filling rate in heat stress; Biotic stress tolerance (FHB, leaf	(Bhusal *et al.*, 2017; Häberle *et al.*, 2009; Qi *et al.*, 2016)
2.	*Xcfd32*	1DL	363.7	CTD1	2.6	-0.37	10.1	TraesCS1D01G267800.1	Ion transport protein (Potassium channel); transmembrane protein; cyclic nucleotide binding	Thousand kernel weight, Seed area & perimeter	(Jing-lan *et al.*, 2015)
3.	*Xbarc124*	2DS	5.2	CTD1	2.9	0.30	11.2	TraesCS2D01G010300.1	Arginine/serine-rich splicing factor; nucleic acid binding; RNA recognition motif	CTD, Biotic stress (leaf rust)	(Kumar *et al.*, 2021; Sharma *et al.*, 2020)
4.	*Xgwm484*	2DS	48.2	SPAD4 NDVI 2 NDVI3	2.7 2.7 2.5	-6.29 -0.02 -0.03	15.9 12.7 13.0	TraesCS2D01G096000.1	MYB transcription factor; Myb-like DNA-binding domain	Several yield attributing traits including yield and harvest index in drought conditions	(Dodig *et al.*, 2012; Zorić *et al.*, 2012)
5.	*Xcfd50*	2DL	637.4	NDVI2 NDVI 3 100SW	2.6 3.0 4.6	0.02 0.05 0.21	12.4 15.3 15.4	TraesCS2A01G559900.1	Transmembrane protein; unknown function	Yield under drought conditions; Biotic stress (Powdery mildew)	(Czyczyło-Mysza *et al.*, 2019; Lu *et al.*, 2012)
6.	*Xcfd35*	3DS	43.4	RWC	3.2	-2.96	18.4	TraesCS3D01G085800.1	Trehalose 6-phosphate phosphatase; Salt	Heat Susceptibility Index (HSI)	(Mason *et al.*, 2011)
								TraesCS3D01G086100.1	Cytochrome P450; iron ion binding		

7.	*Xwmc47*	4BS	644.9	CTD1	3.2	-0.10	12.2	TraesCS4B01G353200.1	Oxygen-dependent choline dehydrogenase;	Yield and root traits in drought	(Kadam *et al.*, 2012)

Position (Mb) - The physical position of marker projected on IWGSC Chinese Spring RefSeq ver. 1.0 reference genome is shown in Megabase pairs. PVE – percent phenotypic variation explained by the marker; CTD – Canopy temperature depression °C (CTD1 – CTD at 50% flowering stage); SPAD – Chlorophyll content by SPAD (SPAD4 - SPAD at 30 days after 50% flowering stage); NDVI – Normalized difference vegetation index (NDVI2 - NDVI at 10 days after 50% flowering stage, NDVI3 - NDVI at 20 days after 50% flowering stage); RWC – Relative water content (%), 100SW – 100 Seed weight

DISCUSSION

In the present study, CTD and SPAD correlated with each other and had a significant positive association with biomass and GY. An increase in BY is significant as it positively correlates with GY (MASON *et al.,* 2011; TSHIKUNDE *et al.,* 2019) and is verified in the present investigation for all the studied crosses. Only with the increase in biomass, a part can be partitioned into GY though an improved HI (TSHIKUNDE *et al.,* 2019). All the crosses for CTD and SPAD deviated from the additive-dominance model, and duplicate gene action was observed for a few crosses. The duplicate gene action was further verified from the observed additive effects of associated markers, particularly for CTD and NDVI observed with both positive and negative additive effects signifying that the favourable alleles are not concentrated in any parent. Altogether deviation from the additive dominance model for CTD and SPAD, signified difficulty in early improvement of these traits and hence recommended selection to be exercised at later generations of segregation.

Non-significance of all the four scales in one or few crosses for RWC, 100 SW, BY, and HI, as observed in the present study, signified the absence of epistasis. This suggested for intra-locus additive/dominance gene effects playing a major role in governing these traits and hence the possibility of an early generation selection for their improvement. The traits following simple additive-dominance model with an absence of epistasis had been reported in the past for RWC (IJAZ *et al.,* 2013), 1000 grain weight (ERKUL *et al.,* 2010), and HI (ABBASI *et al.,* 2013). Barring a few exceptions, rest all the traits in most of the crosses had epistasis, signifying that the early generation selection is not possible and breeder has to delay selection to the later generation of segregation so that homozygosity is fixed and selected. Significant epistatic interactions found in the present study like dominance x dominance [l] interaction for RWC (SAID, 2014), epistatic interactions for HI (ABEDI *et al.,* 2015), all types of digenic as well as duplicate gene action for BY and GY (KAMALUDDIN *et al.,*2007; PRZULJ and MLADENOV, 1999; SAID, 2014) had been reported previously. Moreover, the observation of both additive x additive [i] and dominance x dominance [l] epistatic interactions for most studied traits suggested utilizing hybridization schemes like diallel selective mating, biparental mating, which exploits both additive and non-additive gene action. The genetic controls for a few traits like RWC, 100 SW, BY & HI varied with the crosses and were governed with or without epistasis. This inconsistency in genetic control, be it for presence or absence of epistasis for grain weight, GFP, GY (PATEL *et al.,* 2018; SAREEN *et al.,* 2018) or conflicting reports for duplicate or complementary gene action for chlorophyll content (LJUBIČIĆ *et al.,* 2016; SALMI *et al.,* 2019), RWC (RAVARI *et al.,* 2017), GY (RAIKWAR, 2019) has been observed previously. Thus, it is crucial to consider the dependency of

the gene effect upon the type of genetic material used as parents and the environmental conditions of growth (SALMI *et al.,* 2019). For 100SW, one cross showed non-epistasis, and the other two crosses showed significant additive x additive[i] epistatic interactions. Significant additive gene effect [d] and additive x additive [i] gene interaction for grain weight has been observed in the past (SINGH *et al.,* 1998), which suggests that selection may help improve 100SW as the additive component of variation responds to selection. The fact was further corroborated by associated markers that exerted only a positive additive effect (0.06 -0.21), signifying that only one parent, i.e., UP2828, supplies favourable alleles in the progeny. Altogether the results suggested the possibility of higher genetic gain for 100 SW than other traits of the study.

Late sowing of wheat causes terminal heat stress, which results in a decrease in growth and yield of wheat crop (BHUSAL *et al.,* 2017; PATIL *et al.,* 2012; PINTO *et al.,* 2010). In the present investigation, SSR markers associated at different growth stages post-flowering in heat stress conditions. Interestingly, all these markers have been associated with genomic regions imparting heat or drought tolerance in the previous studies, though to the different traits compared to the one presently identified. It signifies the importance of underlying candidate genes involved in imparting biotic/abiotic stress tolerance via the plant multiple potential pathways. Significant markers were associated with all three radiometric traits. Among the three markers associated with CTD, *Xbarc124* had been previously associated with CTD (SHARMA *et al.,* 2020), and the other two (*Xcfd32* & *Xwmc47*) are the novel markers associated in the present investigation. The candidate gene identified for *Xcfd32* is a transmembrane protein involved in ion transport (potassium channel), which may play an important role in drought sensing and osmoregulation during the water deficit conditions (WANG *et al.,* 2013). The putative gene linked to *Xwmc47* is a choline dehydrogenase having oxidoreductase activity that may be involved in the glycine-betaine pathway, and its role in protecting plants against various abiotic stresses (drought, cold, salt etc.) had been established in the past (HE *et al.,* 2011). Interestingly, *Xwmc47* previously had been associated with several yield and root traits in drought conditions (KADAM *et al.,* 2012). The association with root traits implies higher water extraction ability to keep a cooler plant canopy (TRICKER *et al.,* 2018). Hence, the presently associated *Xwmc47* marker is having a pleiotropic effect on root traits and canopy temperature. Markers *Xgwm484* and *Xcfd50* associated with SPAD & NDVI at different stages of post-flowering. Both of these markers had been associated with yield traits in drought conditions. The putative candidate gene related to *Xgwm484* is a *MYB* transcription factor, which is reported to enhance tolerance to heat & drought in several plant species, including wheat (e.g., *TaMYB30-B*), mainly by regulating drought-responsive genes (GAHLAUT *et al.,* 2016; KADAM *et al.,* 2012). Among the marker associated with 100 SW, *Xwmc728* was linked to a gene coding for cysteine protease-like protein. Abiotic stresses are reported to enhance cysteine protease activity resulting in premature senescence and seed protein degradation (BOTHA *et al.,* 2017). Thus, the presently associated *Xwmc728* marker can help identify plants with less affected protein quality and seed weight. Marker *Xcfd35* associated with relative water content in the present study was previously associated with the heat susceptibility index (MASON *et al.,* 2011). The identified underlying genes are important in photosynthesis and sugar metabolism, helping delay drought stress (LAWLOR and PAUL, 2014). Markers associated with heat, drought or their combined stress in

past studies were highly influenced by the environment with high GXE interaction, highlighting the problem of identifying a stable QTL, especially for physiological traits (TRICKER *et al.,* 2018). Though various researchers report stable QTLs, they also highlighted and reported the identified QTL for a particular environment (PATIL *et al.,* 2012; PINTO *et al.,* 2010). Thus, the associated markers in the present investigation, though identified at a single location with segregating mapping population, might be useful with repeated validation and in similar environmental conditions.

Grain yield per plant is the ultimate result of the interaction of all physiological traits. The present study showed the presence of epistatic gene interactions in all the studied crosses along with duplicate gene action. The presence of epistasis and duplicate gene action had been reported for GY (PATEL *et al.,* 2018; SINGH *et al.,* 1998). Thus, for improving GY, breeder has to wait for later generations so that non-additive gene action diminishes down and additive gene action fixes up, so that better yielding plants can be selected. Positive correlations of grain yield per plant with CTD, SPAD, 100SW, BY and HI in the present investigation indicate the possibility of yield enhancement by incorporating physiological traits in the breeding programme. Positive association of grain yield with CTD, SPAD, 1000-grain weight, biological yield and harvest index had been reported in the past (MASON *et al.,* 2011; POZO *et al.,* 2016; REYNOLDS *et al.,* 1994). Moreover, the present study clearly demonstrates that the studied traits, including the radiometric traits, affect the GY indirectly by enhancing the BY. Thus, the direct or indirect correlations of various physiological traits with grain yield found in the present investigation can be utilized for selection in segregating generations.

CONCLUSION

Correlation and path studies in the present investigation have clearly shown that the physiological traits, especially radiometric traits like CTD, SPAD can improve grain yield directly or indirectly via improving the biological yield. Almost for all the traits, significant digenic interactions either with significant additive x dominance [j] or dominance x dominance [l] interactions were found. Moreover, duplicate gene action was found for all the traits except for the GFP and 100SW in the present study. The results signify that improving the studied traits in the early generation of segregation is not easy, and the breeder has to wait for a few generations of segregation to pick useful plants. However, 100SW can be considerably improved due to the higher magnitude of additive epistatic interaction. Seven different SSR markers i.e., *Xwmc728, Xcfd32, Xbarc124, Xgwm484, Xcfd50, Xcfd35* and *Xwmc47* were associated with the studied traits at different phenological stages in the F_3 population of a cross phenotyped in heat stress conditions. The putative functions identified for the associated markers ranged from the nucleic acid binding domains, transmembrane proteins involved in ion transport, and enzymatic activity important for providing abiotic stress tolerance to the wheat plant. Associated markers in the present investigation have validated a few previous studies, and these markers can be validated further to be used in marker-assisted breeding for wheat crop improvement.

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GENETIČKA ANALIZA I POVEZANOST MARKERA SA FIZIOLOŠKIM OSOBINAMA U USLOVIMA TOPLOTNOG STRESA KOD JARE PŠENICE (*Triticum aestivum* L.)

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Izvod

Utvrđivanje interakcije gena i markera povezanih sa fiziološkim osobinama, posebno u kasnijim fazama nalivanja zrna, može pomoći u razvoju efikasne metodologije oplemenjivanja pšenice. Šest generacija (P_1, P_2, F_1, F_2, BC_1P_1 i BC_1P_2) četiri različita ukrštanja jare pšenice (tolerantna na sušu x osetljiva na sušu) i generacija F_3 jednog ukrštanja, tj. MACS6272 x UP2828 su fenotipizirani i genotipizirani sa ciljem utvrđivanja delovanja gena i povezanih markera. Utvrđena su značajna variranja u opadanju temperature sklopa (CTD - 2,6 - 5,6° C), sadržaju hlorofila utvrđenom korišćenjem SPAD aparata (39,6 - 51,3), relativnom sadržaju vode (RWC - 51,5 - 75,4%), periodu nalivanja zrna (GFP - 61,1 - 80,1 dana), masi 100 zrna (3,7 - 5,5 grama), žetvenom indeksu (HI - 25,8 - 46,2%), biološkom prinosu (BY - 35,5 - 89,8 grama) i prinosu zrna (GY - 13,4 - 36,5 grama) u šest ispitivanih generacija. GY je pozitivno korelirao sa CTD, SPAD, 100SW, BY i HI (0,08 * - 0,85 **). BY je imao maksimalan direktan (0,82) i indirektni efekat putem ostalih osobina na GY. Pronađene su značajne neaditivne epistatičke interakcije (j & l) i duplirano delovanje gena za većinu svojstava, osim za GFP i 100SW. Procenat objašnjenih fenotipskih varijacija (PVE) kod sedam različitih SSR markera povezanih sa CTD, SPAD, NDVI, RWC, 100SW kretao se u rasponu od 10,1% do 18,4%, a marker Xcfd35 objašnjava najviši PVE za RWC. Identifikovani kandidat geni (*in silico*) pripadali su transmembranskim proteinima (Xcfd32, Xcfd50), domenima vezivanja nukleinskih kiselina (Xbarc124, Xgwm484) i enzimatskoj aktivnosti (Xcfd35, Xwmc47, Xwmc728) uključenim u mehanizme tolerantnosti na abiotski stres. Složeno nasleđivanje koje je dešifrovano u šest generacija ukazuje na odlaganje selekcije u kasnije faze segregacije radi poboljšanja prinosa zrna pšenice.

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