Genetic basis of some physiological traits and their association with yield in pre-breeding germplasm of bread wheat (*Triticum aestivm* L.)

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Abstract

The rise in human population necessitates the use of all available tools to enhance wheat productivity. In this regard, pre-breeding has mobilized novel under-utilized genetic variation into breeding programs. However, this germplasm needs to be characterized for its efficient utlization. This investigation was initiated to evaluate the early and late sown wheat pre-breeding germplasm for physiology and yield related traits and to associate the mapped SNPs using association mapping approach. Our results indicate that the germplasm performed better in early sowing in comparison to late planting where grain yield (Yd) was found positively correlated with water use efficiency (WUE), heading time and chlorophyll contents (Chl). We discovered a total of 210 associations involving 155 SNPs. Taking into consideration either early or late sowing and the mean values only 12 markers traits were involved with germination, plant height, stomatal conductance, transpiration rate, Chl, carotenoids and Yd. Our correlations and mapping results indicate that higher WUE along-with Chl can be targeted as indirect physiological markers to enhance wheat yield.

Keywords Association mapping . BreadWheat . Pre-breeding germplasm. Physiological traits. Biplot

Introduction

Wheat (*Triticum aestivum* L.) is the most important crop of the world as global food security heavily relies on it (Curtis and Halford, 2014). A decreasing trend has been observed in global wheat production from last few years owing to multiple factors (FAO 2019). The gravity of the situation is also evident from the fact that until 2050, 9 billion global inhabitants will be served from the same produce. Therefore, current wheat production should be doubled to meet the growing demand by 2050 (Ray et al. 2013).
According to a recent estimate, Pakistan stands 8th in terms of area and 29th in terms of wheat yield per unit area (Hassan et al., 2020). The rise in human population necessitates plant breeders to utilize and adopt all available tools to enhance wheat productivity on a sustainable basis (Curtis and Halford, 2014).
Pre-breeding is playing a pivotal role by mobilizing novel under-utilized genetic variation into breeding programs through introducing less undesired linked genes (Moore 2015). In this regard, Seeds of Discovery (SeeD) project at CIMMYT has resulted in the creation of new pre-breeding germplasm with a view to enhance the use of genetic resources in breeding programs (Singh et al. 2018). The same germplasm has been used to track loci linked to yield-related traits (Akram et al. 2020), nematode resistance (Dababat et al. 2021), karnal bunt (Singh et al. 2020) and terminal drought stress (Shokat et al. 2020a). However, determining the genetic basis of physiological traits has not been attempted so far. Therefore, this investigation was initiated to associate the SNPs with some physiological traits and yield (Yd) in early as well as late sowings of wheat pre-breeding germplasm using association mapping approach.

Materials and methods

The current study was conducted on 118 hexaploid wheat pre-breeding lines derived from vast germplasm of CIMMYT (Singh et al. 2018). **Table S1** provides the pedigree of this germplasm along with genotypic ID. Two plant

experiments were conducted side by side under field conditions; early (24th Oct, 2017) and late planting experiment (24th Nov, 2017). During field preparation nitrogen (N), phosphorous (P$_2$O$_5$) and potash (K$_2$O) were applied at the rate of 60, 90 and 60 kg ha^{-1}, respectively. Seeds were sown manually at the rate of 5 g seeds for 2 meters rows which were 22.5 cm apart. At each of tillering and booting growth stage, N was also applied at 30 kg ha^{-1}. The plants were irrigated 5 times, while the first irrigation was given after four weeks of sowing. All other agronomic and plant protections measures were practiced up to crop maturity.

In total, data regarding 12 morphological and physiological traits were recorded. These included germination % (Gr); recorded three weeks after sowing according to a scale ranging from 0-100%, where 0% = no germination and 100% = complete germination, heading time (Hd); measured when 50% of spikes have emerged from the flag leaf, plant height (Ht); recorded in cm from 5 random plants per replicate through meter rod from the base of plant to spike tip excluding awns, transpiration rate (Tr); calculated using the hand held porometer (Model: LI-1600 steady state porometer) in µg cm^{-2}s^{-1}, stomatal conductance (Sc); in mmol m^{-2} s^{-1} using the formula: 1/DR x CF, where DR is diffusible resistance calculated from porometer, CF = correction factor calculated using the formula: LT x constant (411.8), and leaf temperature (LT) calculated from porometer, photosynthetic rate (Phr); determined from Sc and Tr of three randomly selected flag leaves per genotype using the formula: Phr = Sc (mmol m^{-2} sec$^-$/Tr (µg cm^{-2} s^{-1}) x 10, water use efficiency (WUE) was calculated by dividing the corresponding photosynthetic rate with transpiration rate times 100. Sc, Tr and Phr were recorded separately on sunny days at the time of heading in respective sowings between 11:00 am and 2:00 pm and yield (Yd); recorded after manual threshing in grams per genotype.

Furthermore, same flag leaf samples used for measuring Sc, Tr and Phr were collected from each genotype to measure pigments (chlorophyll and carotenoids). Fresh leaf samples were chopped (0.5 g fresh weight) and extracted with 20 mL 80% acetone (*V/V*). After the addition of acetone to chopped leaf samples glass tubes were placed at 10 °C in dark until complete color disappeared. Afterward, samples were centrifuged at 14000 x g for 5 minutes and absorbance of supernatants was measured at 645, 652, 663 and 480 nm on spectrophotometer (Hitachi-2800) according to Arnon (1949) and Davies and Taylor (1976). From the absorbance readings, chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were calculated using following formula;

$$Chlorophyll\ a\ (Chla) = \frac{[12.7(OD\ 663) - 2.69(OD\ 645)] \times V}{1000 \times W}$$

$$Chlorphyll\ b\ (Chlb) = \frac{[22.9(OD645) - 4.68(OD\ 663)] \times V}{1000 \times W}$$

$$Total\ Chlorophyll\ (ChlT) = \frac{[20.2(OD\ 645) - 8.02(OD\ 663)] \times V}{1000 \times W}$$

Where V is the volume of sample extract (mL), OD is the optical density and W is the weight of fresh tissue.

$$Caroenoids\ (Car) = \frac{A^{car}}{E_{max}\ 100}$$

Where $A^{car} = (OD\ 480) + 0.114(0D663) - 0.638\ (OD645)$ and $E_{max}100 = 2500$

All statistical analyses of phenotypic data including descriptive statistics and visualization (phenotypic histograms, biplot construction and correlation plot) were carried out in RStudio-1.0.153 software. Individual traits measured in early and late sowing and their mean were used to find the marker traits associations (MTAs) associated with traits under study employing freely available software, *TASSEL v5.2.43* (Bradbury et al., 2007). The genotypic data consisting of 7,180 high quality SNPs obtained from Singh et al. (2018) employing PCA matrix (PC=3) coupled with the K-matrix, both of which were generated through *TASSEL v5.2.43*. SNPs that gave a *p*-value of 0.001 (-log10 value of 3) for a given trait were claimed as significant association.

Results and discussion

All the measured traits of the wheat germplasm exhibited continuous variation (**Fig. 1** and **Fig S1-S11**). Higher values recorded in early sowing provide evidence that late sowing showed a negative effect on all the traits except Gr (**Table S2**). Interestingly, Phr was similar in both sowing times. Superior performance in early sowing over late sowing could be due to prolonged growing season (~ 2 weeks) beside other advantages as early flowering in late sowing caused early Hd (**Table S2**) that reduced the assimilates time to mobilize and contribute to plant development and ultimately yield (Rehman-Arif et al. 2020; Bennett et al. 2012).

In order to explore the link of Yd with other traits, combined correlation of both early and late sowing data was performed (**Fig S12**) which indicated that Yd was positively correlated with WUE ($r^2 = 0.25$), Hd ($r^2 = 0.21$), Chla ($r^2 = 0.17$), Chlb ($r^2 = 0.13$) and ChlT ($r^2 = 0.15$). Furthermore, a biplot (**Fig 2**) using PCA was constructed to get latent relationship among the observed traits. As evident from **Fig 1**, Yd was very close to WUE confirming the results of correlation analysis. Previous reports indicate that higher Phr helps plants to maintain optimum grain yield as well as adaptability (Shokat et al., 2020b, 2021). In the current study, Phr was clustered closer to WUE and Yd (**Fig.2**) indicating maintenance of Yd could be due to higher WUE and Phr. It is further suggested that these two parameters can be used as physiological markers to enhance Yd of wheat. However, further in depth investigations are required to explain the hidden mechanism Yd in relation to of WUE.

Association analysis identified a total of 210 associations involving 155 SNPs on all wheat chromosomes except chromosomes 4D and 7D (**Table S3**). Taking into consideration either early or late sowing and the mean values, only 12 markers were identified that were linked with Gr (*M7873* on chromosome 1B at 103.58 cM, $R^2 = 0.10-0.11$), Ht (*M9057* (on chromosome 2B at 165.39 cM), *M4664* and *M3592* (on chromosome 5B at 102.15 and 209.79 cM, $R^2 = 0.12-0.14$), Sc and Tr (*M2654* on chromosome 3B at 81.47 cM, $R^2 = 0.11-0.13$), Chla (*M8777* on chromosome 1B at 114.75 cM, $R^2 = 0.14-0.17$), Chlb (*M12* on chromosome 6A at 97.09 cM, $R^2 = 0.12-0.15$), ChlT (*M9193* on chromosome 1B at 167.58 cM, $R^2 = 0.10-0.11$), Car (*M6457* (on chromosome 2A at 242.24 cM) and *M4336* (on chromosome 2D at 270.96), $R^2 = 0.11-0.14$) and Yd (*M7113* and *M5797* (on chromosome 1B) at 230.06 and 241.77 cM, $R^2 = 0.11-0.13$). Using the same criteria, no MTA was discovered for Hd, Phr and WUE. Rehman Arif et al. (2020) have reported chromosome 1B as carrier of Gr QTLs in NIAB field conditions. Likewise, chromosome 2B is reported to carry Ht MTAs by Akram et al. (2020) at 147-158 cM. Furthermore, chromosome 1B has been focused by Akram et al. (2020) regarding Yd in another set of PBLs. On the other hand, MTAs linked with Chl and other physiological traits do not match with previous reports (Colasuonno et al. 2017; Panio et al. 2013; Zhang et al. 2009) suggesting that a wide range of variation exists in the PBLs with respect to these traits and more investigations are needed to find stable associations across wide range of environments.

Plant growth depends on photosynthesis (Czyczyło-Mysza et al. 2013) and the higher Phr could improve plant output (Shokat et al. 2020b, 2021). ChlT is a trait that can be used as a reliable indicator for photosystem PSII efficiency (Vijayalakshmi et al. 2010). Flag leaf Chl contents at flowering time have been reported to be positively associated with yield in wheat (Quarrie et al. 2006) which supports our finding. Likewise, Car contents are essential for photosynthetic process and in photoprotection of photosynthetic membranes against the large amounts of solar energy absorbed by photosynthetic pigments (Asada 1999). However, our analysis showed no association of Car contents with Yd yet, in biplot, both Yd and Car were going in the same direction. Perhaps Car contents contributed towards Yd but clear mechanism is unknown.

It is concluded that a wide variety of loci in the germplasm are regulating important morpho-physiological traits in wheat. In addition, WUE and Phr can be targeted as indirect markers of selection to enhance grain yield.

Author's contribution Mian Abdur Rehman Arif conceived the idea and designed the study. Maryam, Muhammad Imran and Muhammad Qandeel Waheed performed the experiments. Sukhwinder Singh and Sajid Shokat performed the analysis. Mian Abdur Rehman Arif wrote the manuscript. Sukhwinder Singh, Muhamamd Imran, Sajid Shokat and Anjuman Arif reviewed the manuscript. All authors read and approve the final manuscript.

Compliance with ethical standards

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Table S3 Marker trait associations detected with MLM for various traits at *p*-value < 0.001. Markers that were significant in either of the treatments and mean are in bold