



Full Length Article

Assessment of Genetic Variability and Direct-Indirect Contribution of Post-Anthesis Traits to the Grain Yield in Bread Wheat (*Triticum aestivum*) at Different Sowing Dates

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Received 25 March 2021; Accepted 11 May 2021; Published 10 June 2021

Abstract

In cotton-wheat cropping system of Pakistan, sowing of wheat is delayed which results in a heavy loss to the farmers. To this end, a two-year field experiment was conducted to i) assess the performance of genotypes under late sowing ii) heritability and genetic association of different traits under normal and late sowing for their use in the breeding programs. Experimental factors included 20 diverse wheat genotypes (16 advance lines and 04 approved varieties) factorially combined with two sowing dates *i.e.*, normal sowing (11 and 13 November in 2018 and 2019, respectively) and late sowing (09 and 11 December in 2018 and 2019, respectively). The results revealed a highly significant ($P < 0.01$) effect of genotype and sowing date on all the post-anthesis studied traits and the interaction of the two factors was also significant ($P < 0.01$) for all the traits except number of spikelets per spike. Contribution of sowing dates was more in overall variation of different post-anthesis traits than genotypes and their interaction. With respect to the performance, genotype 9725 performed best in both early and late sowing, but a reduction of 26% in the grain yield was observed due to late sowing, whereas the grain yield of Miraj-2008 was comparatively lower but showed a reduction of only 17% due to late sowing. Number of tillers per plant and biological yield showed a highly significant ($P < 0.01$) correlation ($r = 0.38$ and 0.72 , respectively) with grain yield under normal conditions, but path analyses revealed that number of tillers per plant showed association indirectly through biological yield and its direct effect is only one third of the total effect. Under late sowing, association of number of tillers turned non-significant and direct effect become negative which showed complicated association of this trait under normal and late sowing. High broad sense heritability ($H^2 > 75\%$) of all the traits suggests a good genetic gain in breeding program by using these traits. As different genotypes performed best for different yield related traits, therefore a breeding program with a careful crossing plan should be developed to pyramid the genes for different traits for the development of genotypes adapted to late sowing. © 2021 Friends Science Publishers

Keywords: Late sowing; Wheat breeding; Path analysis; Heritability

Introduction

Wheat is the major cereal crop of the world and used as a staple crop to feed the peoples of the world. It occupies the central position in the global cropping pattern and also in the agricultural policies. It dominates all the crops due to its extensive cultivation and higher production. Wheat is a photoperiod sensitive crop and its reproductive phase starts in late February and early March and matures in the late April to mid-May irrespective of sowing dates especially in plain areas of South Asia. In late sown crop, temperature is low, which results in poor and delayed germination (Shah *et al.* 2019) and results in poor crop stand. Moreover, late

sown plants can accumulate less biomass compared to early grown crop at the onset of reproductive phase due to a shorter crop cycle (Tahir *et al.* 2009; Porker *et al.* 2020) which results in less photosynthetic assimilates and lower yield. Additionally, increasing in the temperature at the onset of reproductive stage of late sown wheat shortens the crop duration (Mo *et al.* 2016) resulting in less assimilates production for accumulation in the grains. Due to specific cropping patterns of the area, farmers face lower yields which not only results in lower profitability of the poor farmers but also effects on the global food security. Even some farmers are replacing wheat with some other non-food crops to get maximum profits (Gaba 2018) and putting

pressure on food security. But on the other hand, higher production of staple crops is essential to feed the ever-increasing world population.

Late sowing of wheat significantly affects plant height, tillering, spikes per plant, spikelets per spike and grain weight which ultimately leads to lowers yield (Wajid *et al.* 2004; Sial *et al.* 2010). It is estimated that delayed sowing (late December) results in the loss of up to 38% reduction in the grain yield (Baloch *et al.* 2012). Late sowing of wheat affects grain yield and its components by affecting crop growth rate due to frost injury at germination stage (Shah *et al.* 2019) and heat stress at booting and spike initiation stage (Ihsan *et al.* 2016). Furthermore, heat stress at anthesis of late sown crop disturbs plant physiological and biochemical processes and results in poor growth with a short life cycle. Telfer *et al.* (2018) conducted a multi-environment experiment to investigate the effect of high temperature during anthesis and grain filling period and reported that with increase in each °C after 30°C resulted in a reduction of 302 kg ha⁻¹ and 161 kg ha⁻¹ at anthesis and grain filling stages, respectively. They further suggested to using field trails for the evaluation of heat tolerant germplasm. Talukder *et al.* (2014) reported that heat stress at heading stage shorten the post heading duration of crop which results in the reduction of final grain yield. Variation in the genotypes in response to late sowing for yield and other components have been well documented (Tahir *et al.* 2009; Talukder *et al.* 2014; Telfer *et al.* 2018; Porker *et al.* 2020). But only a little effort has been placed to select the traits which response best to late sowing for selection of wheat genotypes. Exact knowledge of plant architecture, its response to environmental conditions and its heritability is necessary for development of environment responsive genotypes.

Knowledge of post anthesis plant architecture (Senapati and Semenov 2019; Ullah *et al.* 2020) and association of morphological traits especially yield components with final grain yield (Talukder *et al.* 2014; Ul-Allah *et al.* 2018) is very important in developing specific environment responsive genotypes because plant changes its morphological traits in response to environmental stress. By this we can assess contribution of different traits to the yield and their sensitivity to environmental stress like heat stress in case of late sowing, which will help in selection of the best genotypes. Although there are many studies which describe the effect of late sowing on yield and its components, but there is lack of knowledge about the plant maturity traits, association of the post anthesis traits with grain yield and their direct and indirect effects for the development of the genotypes best adopted to late sown conditions. Moreover, a little is known about the heritability's of different traits of the plant architecture adapted to specific (timely or late) sowing date. Therefore, objective of this study was to investigate the effect of late sowing on the traits of mature wheat plant and their association with final grain yield along with direct and

indirect effects. This study could help to facilitate the optimal selection criteria for development of wheat genotypes adapted to late sowing.

Materials and Methods

Experimental site

The field experiment was conducted in the experimental area of the College of Agriculture, Bahauddin Zakariya University, Bahadur Sub-Campus Layyah, Pakistan during the wheat season of 2018–19 and 2019–20. The experimental soil was characterized as sandy loam with a bulk density of 1.29 Mg m⁻³ and pH was 8.2. The soil has medium fertility *i.e.*, N contents 435 mg kg⁻¹, P contents 6.8 mg kg⁻¹ and K contents 123 mg kg⁻¹. This area falls in a category of subtropical climate with warm summers and cool winter with an average rainfall of ≤ 200 mm. During the experimental seasons, the weather conditions of experimental location were presented in Fig. S1.

Plant material and experimental design

The seed of twenty wheat genotypes (approved varieties and advance lines) was obtained from different research institutes of Pakistan (Table S1) for the first year of crop while, for the second year, self-seed was harvested and reused. The twenty wheat genotypes were sown in two dates of sowing *i.e.*, normal sowing (11 and 13 November in 2018 and 2019, respectively) and late sowing (09 and 11 December in 2018 and 2019, respectively). The experiment was laid out in a randomized complete block design (RCBD) under factorial arrangements with three replications. Factors included 20 wheat genotypes (Table 1) and two sowing dates each in 2018 and 2019. For each genotype, 5 rows, each six feet long were sown with row-to-row distance of 30 cm and plant to plant distance of 15 cm. Five irrigation (pre-sown irrigation, tillering, joint formation, booting and milking stage) were applied to meet the moisture requirements of the crop. Fertilizer was applied at the rate of 120 (N):90 (P):60 (K) kg ha⁻¹ while source of the fertilizer were Urea, Diamonium Phosphate and Sulphate of Potash. Weeds were controlled by applying Buctril Super 60% (Bayer Pakistan Private Limited) EC at tillering stage.).

Data collection

Data were collected from selected 10 guarded plants from each experimental unit. Plant height was measured with the help of a meter rod (soil surface to the tip of awns) of the mother tiller of the selected plants followed by average calculation. From the selected plants, data for number of tillers per plant (counted manually), spike length (with the help of measuring tape, excluding the awns), number of spikelets per spike (counted manually) were recorded. For

biological yield, selected plants were harvested, and sun dried until constant weight and average plant biomass was calculated as an average of 10 plants. After that, the plants were threshed manually, seeds were separated from straw and average grain yield per plant was calculated as an average of ten plants.

Reduction in the performance of different traits of wheat genotypes due to late sowing was calculated by the following formula:

$$\text{Reduction (\%)} = \left\{ \frac{\text{Performance in normal sowing}}{\text{Performance in late sowing}} - 1 \right\} \times 100 \quad (1)$$

Statistical analyses

Collected data were statistically analyzed for analyses of variance followed by comparison of the means by Tukey's test using Statistix 8.1. Genotypic correlation (Fisher 1992) was calculated among yield and yield contributing traits. Direct and indirect effects of correlation were determined by the path coefficient analyses as described by (Dewey and Lu 1959). Standard Broad-sense heritability (Schmidt *et al.* 2019) was calculated for all the studied traits grown under normal and later sowing separately as ratio of genotypic variance to phenotypic variance as given below.

$$\text{Heritability Broad-sense } (h_{b_s}^2) = \frac{GV}{PV} \quad (2)$$

Where, GV and PV stands for genotypic variance and phenotypic variance and calculated as

$$\text{Genotypic variance } (GV) = (MSG - MSE)/r \quad (3)$$

Where, MSG stands for mean square of genotypes and MSE stands for mean square error

$$\text{Phenotypic variance } (PV) = GV + MSE \quad (4)$$

Figures were prepared using Sigmaplot v. 12.

Results

Morphological traits

Analyses of variance revealed that all the traits were significantly ($P < 0.01$) affected by genotypes and sowing dates and the interaction of the two factors was significant ($P < 0.01$) for all the traits except number of spikelets per spike (Table 1). Plant height for normal sowing ranged from 66 (Miraj-2000) to 101 cm (Syn-42) while for late sowing it ranged from 58 (9868) to 82 cm (Syn-46). Maximum reduction in plant height (28%) was observed for the genotype Syn-42 while the minimum was observed in the genotype Syn-31 *i.e.*, 3.4%. Number of tillers per plant for normal sowing ranged from 02 (Miraj-2000) to 05 (Bhakkar-2002) and for late sowing it ranged from 2.6 (Bhakkar-2002) to 3.8 (AS-2002). Maximum reduction for number of tillers was observed in the genotype Bhakkar 2002. Spike length for normal sowing ranged from 8.8 cm (Syn-31) to

12.16 cm (Syn-33) and for late sowing it ranged from 7.2 cm (Syn-40) to 9.7 cm (G 9782). Maximum reduction in late sowing was observed in the genotype Syn-33 (29%) and minimum was observed in the genotype Syn-21 (5%). In normal, sowing number of spikelets per spike ranged from 16 (G Syn-50) to 20 (Genotype 9733) for normal sowing and from 12 (Miraj-2008) to 15 (Syn-33) for late sowing. Maximum reduction (37%) in number of spikelets per spike due to late sowing was observed in the genotype AS-2002 and minimum (19.8%) was observed in the genotypes Syn-33 and Syn-40 (Fig. 1; Table 3).

Biological yield and grain yield were the two traits that were highly affected by the late sowing. In the normal sowing biological yield per plant ranged from 11.5 g (9733) to 20 g (9725) while for late sowing it ranged from 7.7 g (Syn-40) to 15 g (9725). For biological yield maximum reduction (38%) was observed in the genotype Syn-40 and minimum reduction (23%) was observed in the genotype Syn-37. Average grain yield of single wheat plant ranged 4.5 g (G 9733) to 9 g (9725) in normal sowing and 3 g (Genotype 9733) to 5.9 g (Genotype 9782) in late sowing. For grain yield maximum reduction (42%) was observed in the genotype Syn-33 and minimum reduction (25%) was observed in the genotype Syn-31 (Fig. 2; Table 3).

Heritability

Heritability is a statistical measure of inheritance. Broad sense heritability was measured for all the studied traits separately for early and late sown genotypes. Under normal sowing maximum heritability was observed for biological yield (92.88%) and grain yield (92.80%) and under late sowing, it is reduced to 83% and 74%, respectively. But for the spike length and spikelets per spike heritability increased and in late sowing it was 93.12 and 90.12%, respectively (Table 2).

Correlation and path analyses

Correlation coefficients were calculated to measure the association between different traits. It was observed that overall association weakened in late sown wheat. Under normal sowing maximum correlation was observed between grain yield and biological yield ($r = 0.72$) followed by plant height and spike length ($r = 0.63$). Other significant correlations were observed between plant height and number of spikelets per spike, between number of tillers per plant and biological yield/grain yield. In normal sowing negative correlation ($r = -0.18$) was observed between grain yield and spikelets per spike which turned non-significant in late sown wheat. In late sown wheat maximum correlation was observed between grain yield and biological yield ($r = 0.65$) (Table 4).

Path coefficient analysis dissects the correlation into direct and indirect effects. As the value of the correlation coefficients changed over sowing dates, similarly direct and

Table 1: Mean squares of different morphological and yield related traits of 20 wheat genotypes under late and normal sowing dates

Source of variance	df	Plant height	No. of tiller/plant	Spike length	No. of spikelet/spike	Biological yield/plant	Grain yield/plant
Year	1	0.32 ^{ns}	2.1 ^{ns}	0.64 ^{ns}	20.12 ^{ns}	0.24 ^{ns}	1.02 ^{ns}
Sowing date (S)	1	717.90**	136.68**	124.93**	3658.21**	16.38**	824.18**
Genotype (G)	19	31.60**	7.06**	3.33**	279.16**	1.62**	4.14**
G*S	19	11.27**	5.20**	1.964**	64.68**	0.3904**	1.898 NS

SD1 –sowing date 1 (normal sowing); SD-2 –Sowing date 2 (late sowing)

*-Significant at 5% probability; ** significant at 1% probability; ns- non-significant

Table 2: Heritability values (broad sense) of yield and related traits of wheat at two different sowing dates as obtained in 2018-19 and 2019-20

Sowing dates	Plant height		No. of tiller/plant		Spike length		No. of spikelet/spike		Biological yield/plant		Grain yield/plant	
	2018–19	2019–20	2018–19	2019–20	2018–19	2019–20	2018–19	2019–20	2018–19	2019–20	2018–19	2019–20
Year	2018–19	2019–20	2018–19	2019–20	2018–19	2019–20	2018–19	2019–20	2018–19	2019–20	2018–19	2019–20
Normal sowing	93.22	92.54	93.90	91.70	81.94	80.1	93.00	90.94	91.62	88.14	85.14	82.88
Late sowing	84.12	82.36	72.72	77.12	93.08	93.16	86.00	84.12	77.00	73.32	92.24	89.12

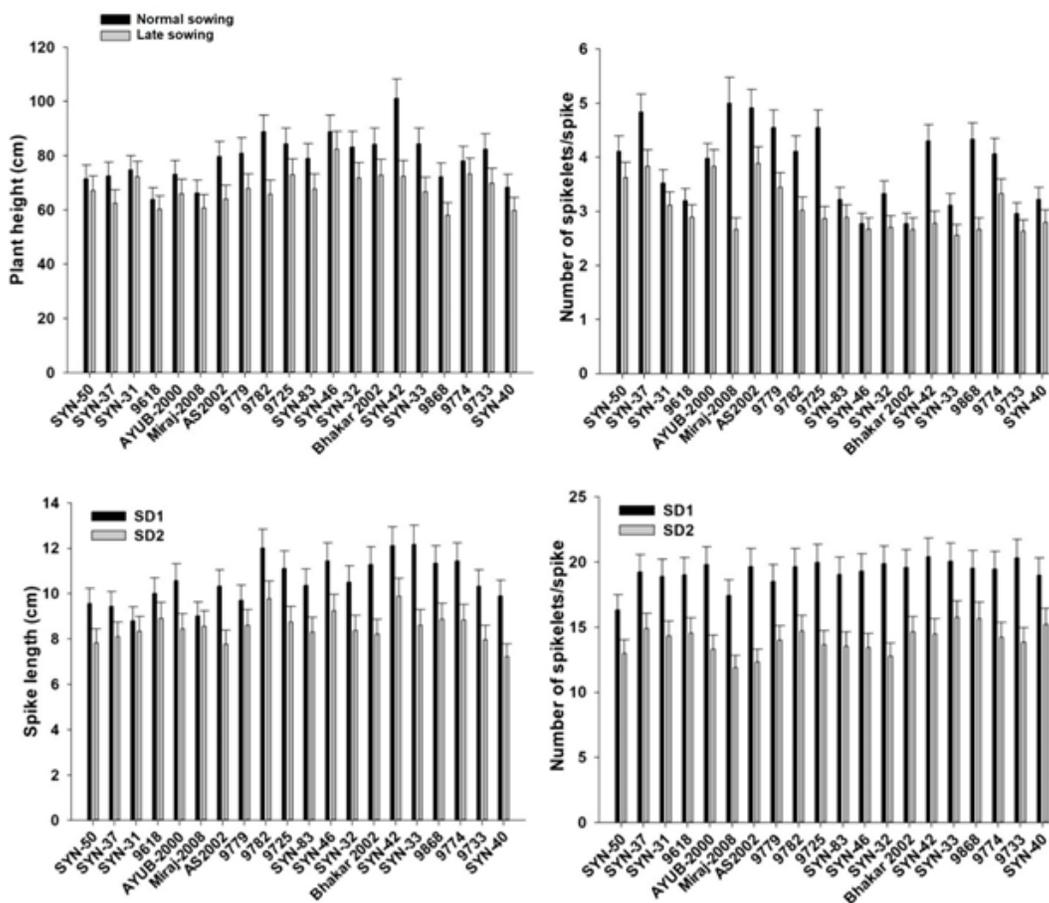


Fig. 1: Effect of genotype and sowing date on different morphological traits of wheat. (Data are average of two years and three replications ± SE)

indirect effects also changed (Fig. 3, 4). Association of different studied traits with yield was dissected and it was observed that most of the indirect effects were very small. Under normal sowing, correlation of number of tillers was 0.38 but path analyses revealed that its direct effect was only 0.12 whereas indirect effect of number of tillers via biological yield was 0.26. Magnitude of other indirect effects was very small and direct effects were relevant to the

overall correlation (Fig. 3). In late sowing, number of tillers had only a very weak association with yield ($r = 0.03$), but its direct effect was -0.11 and its indirect effect via biological yield was 0.11. In late sown genotypes, spike length has a significant association with yield ($r = 0.21$) but its direct effect was only 0.05 and its indirect effect via biological yield was 0.19. Magnitude of other indirect effects was very small (Fig. 4).

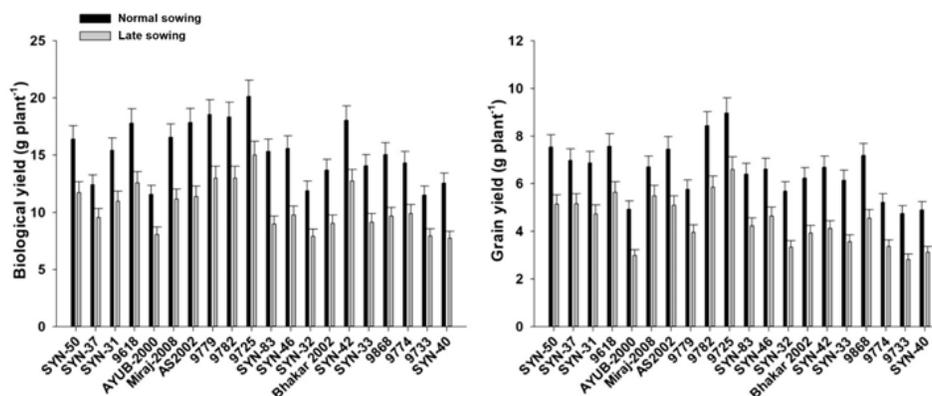
Table 3: Reduction (%) in the performance of various traits of wheat genotype due to late sowing

Traits Genotypes	Plant height	No. of tiller/plant	Spike length	No. of spikelet/spike	Biological yield/plant	Grain yield/plant
SYN-50	6.00	18.26	20.46	11.84	28.50	31.86
SYN-37	13.98	14.12	22.58	20.69	22.99	25.98
SYN-31	3.45	5.31	24.20	11.65	28.86	31.17
9618	5.41	11.00	23.52	9.69	29.40	25.51
AYUB-2000	9.74	20.18	32.66	3.61	30.26	39.46
Miraj-2008	8.38	5.03	31.84	46.67	32.59	17.91
AS2002	19.68	24.69	37.29	20.84	36.20	31.74
9779	16.08	11.24	24.46	24.38	29.93	31.29
9782	25.91	18.59	25.14	26.44	29.13	30.55
9725	13.53	21.24	31.55	37.12	25.53	26.39
SYN-83	14.20	19.94	28.92	10.25	41.51	33.96
SYN-46	7.13	19.23	30.36	3.72	37.29	29.75
SYN-32	13.73	20.12	35.69	18.84	33.56	41.36
Bhakar 2002	13.44	27.11	25.19	3.84	33.95	36.90
SYN-42	28.37	18.39	29.04	35.40	29.39	38.42
SYN-33	20.76	29.26	21.38	17.90	35.00	41.92
9868	19.69	21.76	19.79	38.46	35.69	36.81
9774	6.13	22.81	26.89	17.97	30.88	35.54
9733	15.24	22.80	31.80	10.94	31.13	40.48
SYN-40	12.51	27.14	19.83	13.04	38.42	36.26

Table 4: Correlation among morphological and yield related traits of wheat genotypes under late and normal sowing

Traits	Plant height	No. of tiller/plant	Spike length	No. of spikelet/spike	Biological yield/plant	Grain yield/plant
Plant height	1	-0.15^{ns}	0.46^{**}	-0.08^{ns}	0.01^{ns}	-0.11^{ns}
No. of tiller	0.01 ^{ns}	1	-0.08^{ns}	-0.08^{ns}	0.18^{ns}	0.03^{ns}
Spike length	0.63 ^{**}	-0.09 ^{ns}	1	0.03^{ns}	0.29^{**}	0.21[*]
No. of spikelet	0.40 ^{**}	-0.10 ^{ns}	0.51 ^{**}	1	-0.01^{ns}	-0.09^{ns}
Biological yield	0.17 ^{ns}	0.39 ^{**}	0.06 ^{ns}	-0.15 ^{ns}	1	0.65^{**}
Grain yield	0.06 ^{ns}	0.38 ^{**}	0.04 ^{ns}	-0.18 [*]	0.72 ^{**}	1

Note: *-Significant at 5% probability **-Significant at 1% probability, Bold values = late sowing, non-bold = normal sowing

**Fig. 2:** Effect of genotype and sowing date on biological and grain yield of wheat (Data is an average of two years and three replications \pm SE)

Discussion

In cotton-wheat system, last picking of cotton is done in November, and after the harvest of the cotton sticks, wheat is often sown in December. Growth and production of wheat crop is highly hampered by late sowing due to shorten of growth period coupled with terminal heat stress (Viswanathan and Khanna-Chopra 2001; Dhyani *et al.* 2013; Zain *et al.* 2017; Sisson *et al.* 2018). In current study all the traits were affected by sowing date and genotype but variation due to sowing date was considerably high than

genotypes and their interaction which is evident from the higher mean square values of sowing dates for different traits (Ul-Allah *et al.* 2014). In current study, a significant reduction in the value of morphological traits was observed. As wheat is a photoperiod sensitive crop (Aslam *et al.* 2017; Whittal *et al.* 2018) due to which its growth and development along with size of morphological attributes is highly dependent on duration of crop cycle. Crop of both sowing dates matured in April but difference in the sowing was one month, therefore late sown wheat received less time for growth and development and value of morphological

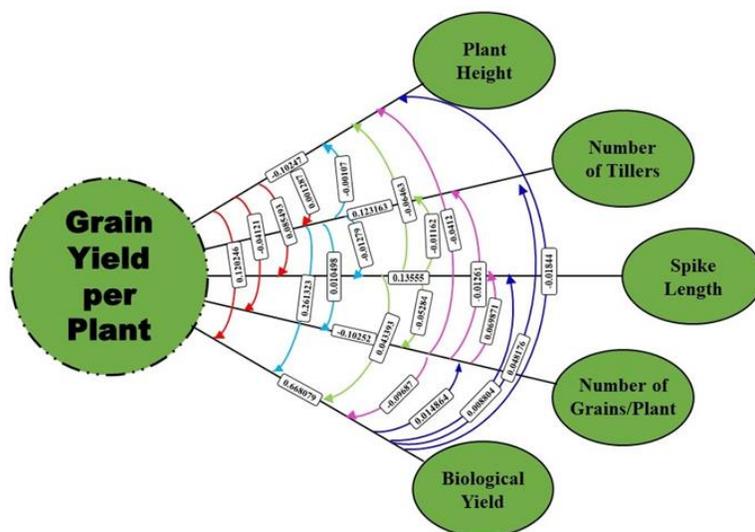


Fig. 3: Path diagram of grain yield different traits affecting grain yield under normal sowing

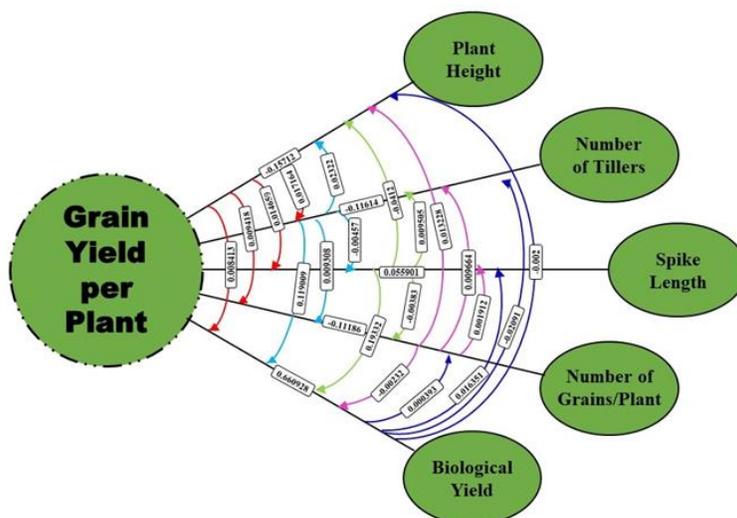


Fig. 4: Path diagram of grain yield different traits affecting grain yield under late sowing

traits remained relatively low. Moreover, in normal sown wheat anthesis completes until the late February, when temperature is moderate, but in late sown wheat anthesis completes in late March where temperature was relatively high and cause less seed setting (Akhter and Islam 2017; Hütsch *et al.* 2019). Hütsch *et al.* (2019) reported that with high temperature, sink capacity of wheat decreased which results in less seed setting with smaller seeds and results in lower harvest index.

In current study, plant biomass and grain yield were reduced which attributed to both *i.e.*, less crop duration and terminal heat stress. As maturity of late sown crop was nearly same as early sown, therefore plant received less time for biomass development and assimilates accumulation (Chakrabarti *et al.* 2011; Whittal *et al.* 2018; Aiqing *et al.* 2018) resulting in lower biomass and

grain yield. But some genotypes resist this reduction in biomass and grain yield to some extent due to better genetic makeup and some possible escape mechanism. Aiqing *et al.* (2018) reported that genotypes escape from terminal heat stress during early reproductive stage by altering their anthesis time and early gametogenesis. They reported 11 genotypes, which flowered during cooler hours of the day to avoid heat stress. Another possible mechanism of relative higher yield of some genotypes under late sowing may be attributed to better nutrient use efficiency (Yin *et al.* 2018) where some genotypes accumulated more assimilate and show less reduction in biomass and grain yield. Therefore, it is suggested that genotypes should be selected very carefully, if the farmers become late in wheat sowing. Moreover, there were different genotypes, which performed best for different traits, which shows

that genes for various traits are present on different genotypes, which needs to be pyramid for development of late sown adoptive genotypes.

For any breeding program knowledge of association among yield related traits and their heritability are very important for the selection of interested traits. Under normal sowing, heritability of all the traits were more than 80% and under late sowing, heritability of only number of tillers was 75% while all other were more than 80%. High value of heritability showed that all the studied traits are valuable for breeding and can result in a significant genetic advance for late sown wheat (Iqbal *et al.* 2017; Mwadingeni *et al.* 2017; Alonso *et al.* 2018; Balkan 2018) but for the selection of traits further studies may be conducted to confirm the gene action governing these traits as Broad sense heritability estimates cannot predict the type of gene action. Alonso *et al.* (2018) also suggested that selection of traits with higher heritability increase the genetic progress in next generations. Heritability of some traits increased and for some traits decrease under late sown which mainly attributed to change in the genetic variation among genotypes in two sowing dates. Change in heritability estimates under different sowing dates showed that selection criteria should be reevaluated for different environmental conditions which are supported by the findings of other researchers working on wheat (Eid 2009; Abou-Zaid *et al.* 2017; Lou *et al.* 2021).

Most of the traits showed weak association among themselves and also with yield, which make the process of selection complicated. Association was also affected by the sowing dates. For example, correlation coefficient of plant height and spike length was 0.63 ($P < 0.01$) under normal sowing while in late sowing it was 0.46 ($P < 0.01$). Likewise, in normal sowing number of tillers were strongly correlated with grain yield ($r = 0.38$; $P < 0.01$), while in late sowing association was non-significant. Differences in the association under different sowing dates attributed the genes which changes their expression under different environment (Eid 2009; Abou-Zaid *et al.* 2017; Lou *et al.* 2021).

As association for some traits were weak and for some other traits, changes over the environment, therefore, these were dissected into direct and indirect effect to analyze how different traits affect yield directly and indirectly by affecting other attributes. Under normal sowing, number of tillers showed strong correlation with grain yield ($r = 0.38$; $P < 0.01$), but path analyses showed that only 1/3 of this association was direct while other attributed to indirect effect mostly thorough biological yield (Fig. 3). Likewise, under late sowing, a non-significant association *i.e.*, number of tillers with grain yield was observed, but path analyses showed negative direct ($r = -0.11$) effect of number of tillers on yield (Fig. 4). These changes, attributed to the activation of different type of genes and change in the association of linked traits under different sowing dates (Abou-Zaid *et al.* 2017; Agrawal *et al.* 2018; Lou *et al.* 2021). Agrawal *et al.* (2018) showed that direct and indirect effects of phenotypic

traits and their association changed under normal and late sown chickpea. Lou *et al.* (2021) reported up to 13% variation in association of different QTLs with phenotypic nutritional quality traits of wheat under two sowing dates and suggested this change in association linked with the genes which expressed only in stress conditions (Muthusamy *et al.* 2017; Mahmoud *et al.* 2020). This change in association and direct and indirect effects necessitate the change in selection criteria for wheat grown on different sowing dates and different environmental conditions.

Conclusion

Late sowing reduced the grains yield and biomass due to short crop duration and terminal heat stress. Change in the association of morphological traits and grain yield under normal and late sowing necessitate the careful modulation of selection criteria for different environments even at same location. As most of the traits showed significant indirect contribution, therefore, those traits should be considered for maximum selection gains. As different genotypes performed best for different yield related traits, therefore a breeding program with a careful crossing plan should be developed to pyramid the genes for different traits for the development of genotypes adoptive to late sowing.

Acknowledgements

Authors are thankful to AARI Faisalabad, Department of PBG, university of Agriculture, Faisalabad and Department of PBG, BZU Multan for provision of seed material.

Author Contributions

S. Ul-Allah contributed to the study conception and design. Material preparation, data collection was performed by M. Bibi, A. Azeem and N. Abbas, analyses were performed by S. Ul-Allah, A. Azeem, MA Saleem and A. Sattar. The first draft of the manuscript was written by S Ul-Allah and A Azeem. A Sher, M Ijaz and M Husnain contributed to execution of the study and reviewed and edited the manuscript. All authors approved the final manuscript.

Conflicts of Interest

The authors declare that they have no competing interests.

Data Availability

Not Applicable.

Ethical Approval

Not applicable.

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