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GRAIN YIELD AND QUALITY PARAMETERS OF WHEAT GENOTYPES AS INFLUENCED ACROSS AN ELEVATION GRADIENT

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Abstract

A genotype's yield performance is a genetic expression in an environment. However, other than soil and fertility levels, the elevation gradients also expressed changes in yield traits affecting grain yield and quality. This study was designed to evaluate the performance of wheat genotypes across elevation gradients in terms of yield and quality traits. In the winter seasons 2017-18 and 2018-19, three field experiments were conducted in a randomized complete block design having three replications. Four promising wheat genotypes (Pirsabak-2005, Pakhtunkhwa-2015, Pakistan-2013 and DN-84) along with three candidate lines (P-2, P-12 and P-18) were assessed at the Agriculture Research Farm, University of Agriculture Peshawar (AP), Agriculture Research Station at Kashmir (AK) and Agriculture Research Station at Chitral (AC). Two years' average data across genotypes resulted in the highest tillers density (358 m^2) in AP, followed by AC (346 m^2), and the lowest (256 m^2) in AK. Likewise, grain weight per spike also differed with the highest (2.01g) in AC, followed by AP (1.9g), and the lowest (0.74g) in AK. Grain number was observed the highest (36 $spike^{-1}$) in AP, followed by AC (43 $spike^{-1}$), and the lowest (29 $spike^{-1}$) in AK. Changes in yield traits caused variation in grain yield. Moreover, grain quality differed significantly ($p<0.05$) in the ratio of changes in grain amylose and amylopectin contents. Grain amylose and amylopectin for AP, AK and AC were found to be 22.51% and 77.5%, 25.14% and 74.9%, and 25.83% and 74.2%, respectively. Similarly, the grains gluten and N-content varied by 28.5% and 2.02% in AP, 23.4% and 2.09% in AK, and 25.9% and 2.02% in AC, respectively. Averaged across genotypes, P-18 resulted in maximum tillers numbers and grain number and amylopectin content with relatively a stable amylose and amylopectin ratio for Pakistan-2013. Based on results, it is concluded that elevation gradient is also the governing factor affecting primary traits which expressed different yields with variable grain quality.

Key words: Amylose, Amylopectin, Genotype, Gluten, Grain number, Grain weight, Wheat yield.

Introduction

Wheat (*Triticum aestivum* L.), is the main cereal crop in central Asia and worldwide (Giraldo *et al.*, 2019) as well as in Pakistan (Anon., 2019) and serves as a source of 20% protein and food calories (Giraldo *et al.*, 2019). The protein of wheat is mainly gluten (80%) making it popular for backing bread, noodles, and pasta. Wheat is broader adaptability to multiple environments and shows relatively better resistance to heavy metal stress drought and salinity (Sehgal *et al.*, 2018)). Wheat demand is increasing day by day due to an increase in daily consumption (Feng *et al.*, 2021). In 2050, the world is expected to require 14886 million tons of wheat grains (Islam & Karim, 2019). The yield of wheat highly fluctuates in the countries of Central Asia. The challenge of the changing climate with rising temperature, an unexpected early drought at sowing, water stress during growth, heat and light stress at anthesis and grain development is causing reduced production per unit area (Ali *et al.*, 2021) Drought stress in some parts has adversely affected production (Ullah *et al.*, 2019). Drought at sowing in the rainfed area is and will be a threat that may expand with time (Hussain *et al.*, 2021). The increased temperature could be a blessing in some parts of the country but could be a major risk to the current wheat production (Khaliq *et al.*, 2019). Other than soil and its nutritional status, the climate is the governing factor of plant growth for genotypes performance. It is obvious that elevation gradients play a role in altering the temperature and hence the climate.

The optimum temperature for wheat growth is around 25ºC with a base temperature of 4ºC (Paudel *et al.*, 2021). A rise in temperature from optimum changes the growth mode from vegetative to reproductive as anthesis and grain filling start around 24ºC (Ullah *et al.*, 2019). Temperature rise up to 25 accelerates maturity with rapid changes in development and can impair yield and/or grain quality (Paudel *et al.*, 2021). Severe heat stress during the reproductive stage of the crop may also adversely affect yield (Girousse *et al.*, 2021). Greenhouse gas (GHG) emissions that lead to climate change are other emerging threats to crops in central Asia (Hassan *et al.*, 2022). Wheat plants, after emergence, face dormancy due to winter temperature, which extends with cold intensity in a region. However, growth becomes active with a rise in temperature of spring till it reaches the maximum threshold level to change vegetative to reproductive growth in season (Ceglar *et al.*, 2020). However, a rapid temperature increase may adversely affect yield by attaining early maturity or affecting grain quality (Akter & Islam, 2017). The rise in temperature at anthesis has resulted in a loss in yield (Kamal *et al.*, 2019) by adversely affecting the leaf's photosynthetic activities and accelerating leaf senescence (Goher & Akmal, 2021). Nonetheless, yield changes have been observed differently in different climates, particularly with a 1ºC rise in temperature at the reproductive stage (Akter & Islam, 2017). Adverse effects were reported with high temperatures on biomass with leaf scorching and early senescence (Ullah *et al.*, 2019). A temperature of 24ºC at flowering has caused floret sterility and limited grain by reducing growth duration (Impa *et al.*, 2021).

The current study aimed to investigate yield traits' effect on overall grain yield as well as grain quality parameters of the potential genotypes (i.e. four varieties and three advanced lines) of wheat planted with the same production technology but with different elevations ranging from 350m to 1880m.

Materials and Methods

Experimental site and location: Field experiments were carried out at three different agro-ecologies across the elevation (E) gradients: i.e. the University of Agriculture Peshawar (AP 350m), the Agriculture Research Station Garhi-Dopatta Kashmir (AK 819m), and the Agriculture Research Station Booni in Chitral (AC 1880m). The AP experimental site is 350m above sea level, with a subtropical climate receiving annual precipitation of about 500-700mm, mean daily temperature varies from 24±6.24°C to 40.7±6.29°C from Nov. to May (Arif *et al*., 2021). United States Department of Agriculture (USDA) classification shows that the soil of the experimental site is halpic luvisol, alkaline clay-loam and contains organic matter of around 1% (Irum *et al.,* 2017). The AK falls within the Himalayan orogenic belt. The experimental site (AK) is 819m above sea level, the climate is high moist temperate, receives annual precipitations of 1242mm. The average maximum temperature varies from 20-32°C and the minimum 4-7°C. Soil is clay-loam, contains sufficient organic matter, available Phosphorus and Potassium. Soil has a pH of 6.5 to 7 (Almas & Saeed, 2000). The AC experimental site in Chitral-Bunni is 1880m above sea level, where the climate is of Mediterranean type with warm summers and severely cold winters with 800mm rainfall annually. The average daily temperature varies from 11.4-32.8°C. Soil texture ranges from silt-loam, slightly acidic and moderate to high calcareous having salinity indication in patches. Soil organic matter is greater than 2% (Ahmad *et al*., 2018). Temperatures (Max. and Min.) as well as rainfall data for both crop seasons i.e. 2017-18 & 2018-19 were obtained from Pakistan Meteorological Department (PMD) and are shown in (Fig. 1).

Experimental design and layout: At each location, all experiments were replicated 3 times using randomized complete block designs by following the common field layout. Treatments were wheat genotypes of which 4 approved varieties (Pirsabak-2005, Pakhtunkhwa-2015, Pakistan-2013 and DN-84) and three exotic lines (P-2, P-12 and P-18) at each location were sown. Pakistan-2013 was released by National Agriculture Research Center (NARC), Islamabad and is recommended for cultivation in all parts of Pakistan. Pirsabak-2005 was released by Cereal Crop Research Institute (CCRI), Pirsabak-Nowshera and is widely accepted for bread. Pakhtunkhwa-2015 was also released by CCRI and is recommended for cultivation in rainfed and irrigated areas within Khyber Pakhtunkhwa (KP). Genotype DN-84 was released in 2017 by Agriculture Research Institute (ARI) Ratta-Kulachi, Dera Ismail Khan and is preferred for grain and high straw yield. The 3 lines were imported from Turkey and performed the best in higher altitudes with taller plants and late maturity.

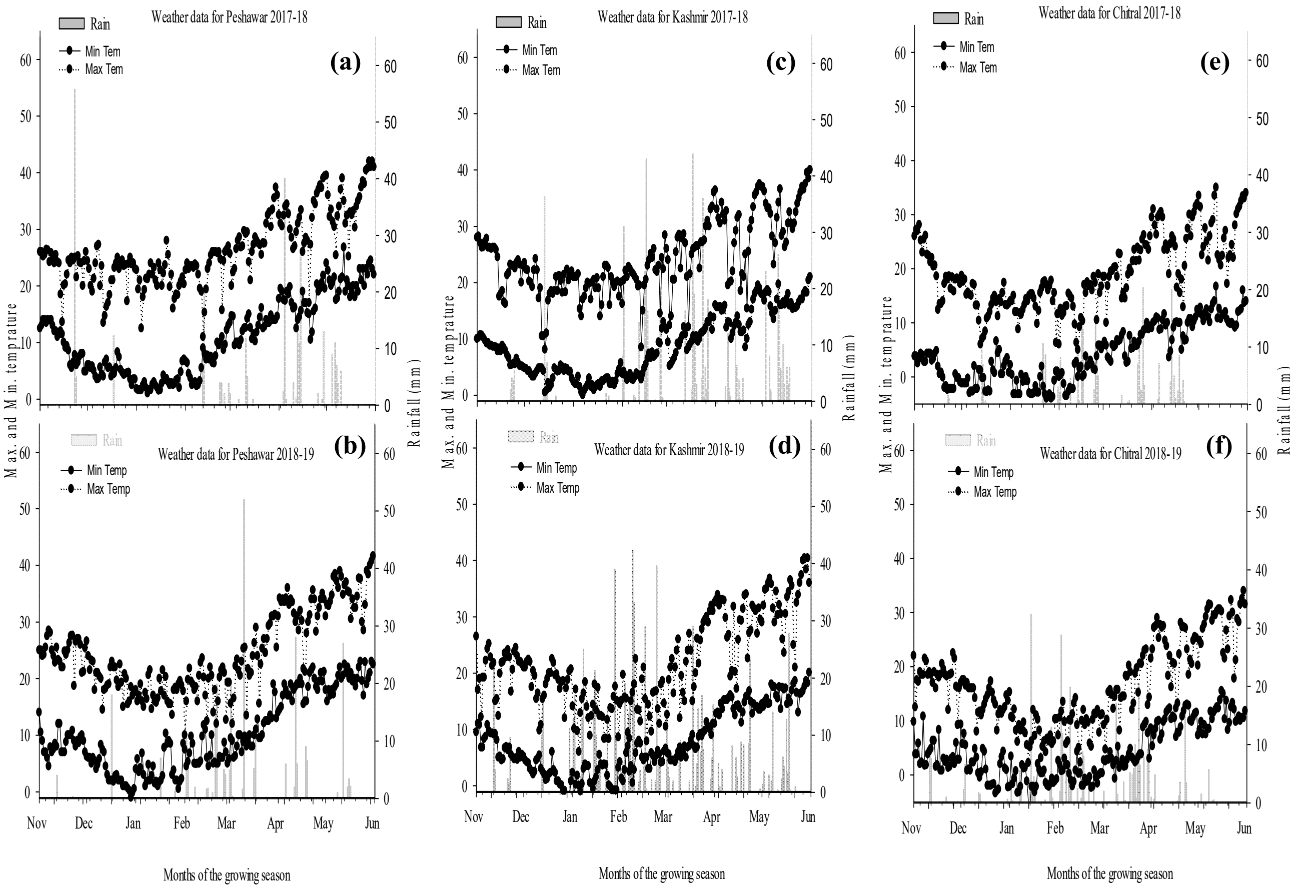


Fig. 1. Data regarding temperature (Min & Max ^{O}C) and rainfall (mm) of the two wheat crop seasons (2017-18 and 2018-19) are in

separate windows for each elevation (a & b), (c & d) and (e & f) for AP, AK and AC respectively.

The crop was sown on November 30 and 10 in 2017 and 2018, respectively in AP, November 27 and 21 during 2017 and 2018, in AK and October 13 and 12 in 2017 and 2018, respectively at AC. For all three experiments, a uniform seed rate i.e. 100 kg ha^{-1} was used at each location. A hand-driven drill was used for sowing by maintaining the 6 rows per experimental unit at a 30cm distance. All experimental units were 5m and 1.8m. Recommended rates of fertilizer N 120 (Urea), P 80 (Single Super Phosphate) and K 50 kg ha^{-1} (Murate of Potash) were applied. All P and K and 50% N were applied at seed-bed preparation, whereas, the remaining 50% N with irrigation i.e. 50 days after sowing (DAS). All cultural operations i.e. weeding and insecticides (Logran) were applied as recommended. Supplementary irrigation, other than natural rainfall, was applied to AP and AC. Harvesting was done on May 4, 2018, for all genotypes but a week thereafter i.e. May 10, 2018 and May 13, 2019, for lines P-2 and P-18 at AP, May 25, 2018 and May 29, 2019, at AK, and July 10, 2018 and July 15, 2019, at AC.

Measurements and observations

Yield traits measurements: Data were recorded on tillers' density per unit area by manually counting tillers in a meter-long row at three locations in an experimental unit and averaged for a single reading. From each experimental unit, 10 spikes were randomly selected close to maturity, dried in a forced-air dryer for 36h, threshed and measured for grain weight and grain number spike^{-1}. After weighing samples, grains were counted on an auto-seed counter for grain weight. Grain yield data were recorded by harvesting two central rows, bundled and dried in fields for 10 days. A mini-lab thresher was used to thresh each bundle and collected grains were immediately weighed using an electronic balance. Grain yield was adjusted with standard grain moisture content (16%) after threshing with the recorded grain moisture content. A handsome quantity of grain samples was preserved for qualitative analysis in a refrigerator.

Quality traits measurements: The preserved grain samples of each experimental unit were dried for 5h in a forced circulating air dryer (70ºC). Grains were ground by passing through a lab grinding mill (Cyclone Mill Twister, 50/60 Hz, UK), at a 2-mm size. Determination of amylose and amylopectin (%) was performed by using the calorimetric iodine method (Juliano, 1971). Details of the procedure adopted for measuring amylose and amylopectin using an amylose curve were the same as those published (Goher & Akmal, 2021). Grain wet gluten (%) was determined by the hand wash method (Anon., 2004). A sample of 20g was added with 14 ml water to form a wet dough. For an hour, the dough was kept untouched and then washed in flowing water in a tub and soluble matter and starches were removed. The resultant gluten ball was kept in a tarred, flat-bottomed dish and weighed as wet gluten. Gluten (%) was determined as the ratio of glutted boll and initial sample weight. Grain N-content was determined using the Kjelflex-K360 (Buchi, Switzerland) following Kjeldahl (Jones, 1991) protocol. Briefly, a 0.2g sample with 1.3g of digestion mixture (20g $CuSO_4$ + 100 g K_2SO_4 + 0.2g Se) was digested along with 3 ml of concentrated H_2SO_4 in a digestion tube. The digest was filtered and distilled water was added, making the volume 100ml. A 100ml sample was run on Kjelfelx-K360 using 40% NaOH (400g NaOH in 1L distilled water) and 4% boric acid (H_3BO_3). The distillate was titrated with 0.1 N HCL using 877 titrino plus and N content was recorded. Reading for total N in a sample was adjusted with blanks, and crude protein was derived by multiplying 6.25.

Statistical analysis

The computer program 'Statistix' was used for the data analysis by following the protocol for the randomized complete block design as per the procedure explained by Fisher's Analysis of Variance Technique. Using the least significant difference (LSD) test means found significant ($p<0.05$) were separated (Steel *et al*., 1997).

Results and Discussion

Yield and its contributing traits

Tiller and grain number, grain weight and yield: Tiller number, grain weight (g) grain number and grain yield (kg ha^{-1}) showed differences ($p<0.05$) within different elevations and genotypes (Tables 1 & 2). Averaged over genotypes, two years means showed differences in tiller number within elevations. The highest tiller number (357.5 m^{-2}) was observed in AP, followed by AC with the lowest (256.1 m^{-2}) in AK. Similarly, the highest grain weight (2.01 g), grain number (43) and grain yield (5386 kg ha^{-1}) was recorded in AC, followed by AP and the lowest (0.75g), (29) and (1979 kg ha^{-1}) in AK. Changes in tiller number might be associated with soil fertility, as mentioned earlier under the heading site and location, where soils of elevation differed significantly. The water content i.e. rainfall and climate situations did differ within the elevations of the study and hence caused differences in tiller numbers per unit area (Harb *et al*., 2020). The other two important traits for yield are the grain number and weight per spike varied ($p<0.05$) differently within elevations. Differences in grain number were associated with spikelet number on spikes and the fertility of florets on spikes (Guo *et al*., 2018). The temperature at the time of anthesis played a critical role in grain growth and development. A difference in the mean daily temperature of an elevation is reported differently which caused differences in grain number per spike (Hussain *et al*., 2021). At the anthesis stage, an increase in temperature has resulted in a lower grain weight, which is probably due to limited assimilates accumulation by a developing ovule (Sehgal *et al*., 2018). Heat intensity and duration played a role in changing the temperature of the day and hence the crop development. Moreover, the high infertility of florets due to abnormal temperature stress has brought ($p<0.05$) changes in grain number and grain weight (Impa *et al*., 2021). Heavier grain weight was reported in AC, which was due to the slow rate of thermal unit accumulation for a limited duration but for an extended period after anthesis for ovule development, which extends the grain fill duration (Ballesteros-Rodriguez *et al*., 2019). Differences (p<0.05) in yield traits at AC and AP from AK were due to soil moisture. According to (Ali *et al*., 2021), a crop with plenty of soil water availability during growth has better grain growth as its escape stress index. It may be due to quick embryogenesis as compared to the crop facing heat stress at the reproductive stage of the crop, which resulted in poor grain formation along with reduced grain number and weight. Limited grain number was observed due to poor pollination caused by water shortages at anthesis (Ali *et al*., 2021) The AK was relatively cooler but rainfed, AP was warm but irrigated and the AC was cool and irrigated with sufficient rainfall, which showed variation in grain number and weight and hence the yield.

Grain yield is the outcome of yield traits, including grain number and weight per spike. It did differ (p<0.05) within three elevations and hence showed variations (p<0.05). The inadequate productive tillers with shriveled grains resulted in the lowest grain yield in AK (Table 2). Unlike AP, the rapid daily decreasing temperature of the crop season in AK has reduced tiller density. Whereas**,** in AC, the crop was sown relatively early in the season, hence resulted in a higher tiller density**.** The reason for variations in sowing dates within the elevations was soil temperature to allow seed germination (Hussain *et al*., 2021) and land free for sowing from the previous crop. The cooler climate of higher elevations delays crop maturity by extending its duration to stay in a cropping system (Khaliq *et al*., 2019). As compared to AP and AK, AC was a mono-crop zone due to limited sunshine hours due to the highest altitude. Appropriate climate under the changing climate delays anthesis and/or maturity with appropriate temperatures for vegetative growth and/or longer grain development phase to reflect healthy traits (e.g. grain number and higher number) to yield more (Khaliq *et al*., 2019) . In the recent past, climate changes i.e. erratic rainfall at crop anthesis stage have created stress at higher temperatures in AP and good growth in AC (Hanif & Ali, 2014). Both soil and climate play significant roles in altering yield traits within different elevations, which resulted in differences in grain yield (Girousse *et al*., 2021).

While averaged across elevation gradients for two years, genotypes differ in their tiller numbers, with the highest (365.7 & 362.8 m^2) in P-2 as well as in P-18, followed by DN-84 and the lowest (289.7 m^2) in Pirsabak-2005 (Table 1). Differences in tiller number within genotypes are both genetic as well as phenotypic. Spacing and climate play a major role in the yield of tiller density per unit. In cereals, tiller density correlates with leaf appearance per tiller (Klepeckas *et al*., 2020). When compared to the years 2017-18 and 2018-19, the maximum tiller number observed in 2018-19 was due to the crop being planted a few days earlier in the season. A few days of early-season crop sown resulted in enough seedling growth to replicate the optimum density (Khaliq *et al*., 2019). A decrease in the temperature during the winter season slows the growth process and hence limits the tiller number per plant affecting low density per unit area within years (Klepeckas *et al*., 2020).

Table 1. Tiller number (m^{-2}) and grain weight (g spike^{-1}) of wheat genotypes planted at different elevations in 2017-18 and 2018-19.

Elevation (E)	Tiller number (m^{-2})			Grain weight (g) spike^{-1}		
	2017-18	2018-19	Mean	2017-18	2018-19	Mean
AP	349.0	366.0	357.5 a	1.01	1.16	1.09 b
AK	254.1	258.1	256.1 c	0.70	0.80	0.75 c
AC	362.2	329.3	345.7 b	2.25	1.78	2.01 a
LSD (0.05) for E	4.57	6.89	3.68	0.18	0.07	0.08
Genotypes (G)						
Pirsabak-2005	287.3	292	289.7 d	1.31	1.26	1.29 b
Pakhtunkhwa-2015	289.4	294.4	291.9 d	1.31	1.25	1.28 b
Pakistan-2013	303.1	309.4	306.2 c	1.55	1.36	1.45 a
DN-84	314.8	315.0	314.9 b	1.33	1.17	1.25 b
P-2	377.8	347.7	362.8 a	1.21	1.1	1.15 c
P-12	302.7	312.0	307.3 c	1.28	1.31	1.30 b
P-18	377.4	354.0	365.7 a	1.26	1.28	1.27 b
LSD (0.05) for G	11.16	8.23	6.81	0.11	0.06	0.06
Year (Y) mean	321.8 a	317.8 b	*	1.32	1.25	*
Significance level (p<0.05) for treatment interaction						
E x Y	-	-	**	-	-	**
G x Y	-	-	**	-	-	**
G x E	**	**	**	**	**	**
Y x G x E	-	-	**	-	-	**

Statistically similar means within a category of treatments are represented by the same letters using the least significant difference

(LSD) test (p<0.05); Significant level * (p<0.05), ** (p<0.01) and NS = Non-significant

Table 2. Grain number (spike^{-1}) and grain yield (kg ha^{-1}) of wheat genotypes planted at different elevations in 2017-18 and 2018-19.

Elevation (E)	Grain number (spike^{-1})			Grain yield (kg ha^{-1})		
	2017-18	2018-19	Mean	2017-18	2018-19	Mean
AP	35	36	36 b	2885	3303	3094 b
AK	28	29	29 c	1791	2166	1979 c
AC	46	39	43 a	5688	5083	5386 a
LSD (0.05) for E	3.7	1.2	1.7	231.7	278.3	161.21
Genotypes (G)						
Pirsabak-2005	33	33	33 cd	3587	3645	3616 b
Pakhtunkhwa-2015	32	32	32 d	3581	3521	3551 b
Pakistan-2013	39	35	37 b	3967	3944	3956 a
DN-84	39	34	37 b	3265	3330	3297 c
P-2	38	37	37 b	3144	3149	3146 c
P-12	35	33	34 c	3459	3744	3602 b
P-18	41	39	40 a	3181	3290	3236 c
LSD (0.05) for G	2.7	1.5	1.5	260.9	223.8	168.9
Year (Y) mean	36.5	34.5	*	3455	3518	NS
Significance level (p<0.05) for treatment interaction						
E x Y	-	-	**	-	-	**
G x Y	-	-	**	-	-	NS
G x E	**	**	**	**	**	**
Y x G x E	-	-	**	-	-	NS

Statistically similar means within a category of treatments are represented by the same letters using the least significant difference (LSD) test (p<0.05); Significant level * (p<0.05), ** (p<0.01) and NS = Non-significant

The maximum grain weight (1.45g spike^{-1}) was observed in Pakistan-2013, thereafter P-12, Pirsabak-2005, Pakhtunkhwa-2015, P-18, and DN-84 with the lowest (1.15g) for line P-2 (Table 2). Genotypes did differ in grain weight due to their sizes, as an accumulation of assimilates per unit time corresponds to the source and climate (Feng *et al.*, 2021). Moreover, food accumulation reserves decreased in some genotypes because of their early maturity, which resulted in smaller and lighter grains. The grain fill process may also coincide with growth temperature to play a role in yielding the optimum grain weight for a genotype (Feng *et al.*, 2021).. Genotype adaptability to a wider range of climates has shown higher grain weight (g) in different altitudes Osman *et al.*, 2020). Pakistan-2013 showed a higher grain weight than others due to its starch accumulation ability at grain development at multiple altitudes. Temperature increase at the grain filling stage has adversely affected grain growth due to its early maturity, hence decreased weight. Similarly, a higher grain number (40) was observed in lines P-18, followed by P-2, DN-84, and Pakistan-2013, which showed statistically the same response but differed from lines P-2 and Pirsabak-2005. Differences in grain number of the spikes are due to changes in grain weight and sizes (Ballesteros-Rodriguez *et al.*, 2019), which caused significant changes in the yield of genotypes of a species. In this study, genotypes P-18, P-2, DN-84, and Pakistan-2013 revealed variation in grain number per spike as compared to the rest of the genotypes. It was due to changes in spikelets number and the rate of infertility of the spikelets. Similar variations in spikelet of spikes under prevailing temperatures played a significant role (Guo *et al.*, 2018). The anthesis stage of the crop is the most critical stage and responds to the temperature accordingly. Temperature fluctuations at elevation, preferably at lower altitudes, adversely affected the grain yield (Osman *et al.*, 2020). Among genotypes, Pakistan-2013 was found to be the leading one with maximum production (3956 kg ha-^{1}) yielding a stable performance from lower to higher altitudes, followed by Pirsabak-2005, P-12, and Pakhtunkhwa-2015, which did not show any change (p<0.05) from each other. The higher yield of a genotype is associated with better trait production in different environments e.g. altitudes (Kamal *et al.*, 2019). The better grain yield of all genotypes in AC was due to the maximum number of days taken by the crop to stay at a relatively lower temperature, as shown by the late harvesting date (Kumar *et al.*, 2019). Nonetheless, Pakistan-2013 resulted in a good yield over other genotypes in all three altitudes, expressing stability in diversified climates for future yields (Osman *et al.*, 2020).

Interaction of E x year (Y) exhibited changes (p<0.05) in tiller number for 2017-18 and 2018-19 with slightly higher in AP in 2018-19, unchanged in AK and slightly higher in 2017-18 in AC (Fig. 2a). The higher tiller number in AP during 2018-19 was due to timely sowing. Similarly, the higher tiller at AC was due to early sowing and a gradual decrease in temperatures of the following days during the growth. Contrary to this, AK was rainfed and the low temperature might have restricted tillering. Treatment interaction (E x Y) also exhibited changes ($p<0.05$) in grain weight and hence the yield (Fig. 2b). Changes in grain weight are noticed in AP and AK with marked differences in AC between years (Fig. 2b). The year 2018-19 showed higher ($p<0.05$) grain weight in AP and AK but lower in AC. Crop took more days in the year 2018-19 in AP and AK due to relatively early sowing in the season. Whereas, higher precipitation at anthesis in AC for the year 2017-18 favored higher grain weight (Fig. 2b). A crop sown early in the season has expressed healthy traits (Klepeckas *et al.*, 2020). Grain number spike^{-1} did not change between years in AP and AK but was higher in AC for 2017-18 (Fig. 2c). As explained earlier, an early sown crop resulted in healthy traits, i.e. grain weight, but higher grains at the anthesis of 2017-18 might adversely affect fertilization and hence affected grain number accordingly (Klepeckas *et al.*, 2020). Grain yield in AC was the highest in both years of the study due to a longer period of growth and development under a mild environment (Fig. 2d). Changes in yield between years are obvious due to changes in the climate (Kamal *et al.*, 2019). A marked decrease in yield was noted in AC to AP with decreasing elevation due to temperature rise that accelerated the growth rate. Contrary to this, the lower yield in AK was due to drought, which adversely affected traits, i.e. tiller number, grain weight, and grain number despite their growth in a cooler climate as growth depends on water exchange by plants with C fixation (Guo *et al.*, 2018).

The interaction of genotype (G) x Y exhibited marked differences within the tiller number, grain weight, and years of the study. A maximum tiller number was observed for genotypes P-2 & P-18 in 2018-19 when compared with 2017-18 (Fig. 3a). The difference between tiller numbers of genotypes in years is obvious due to changes in the sowing time and season of the crop growth after emergence to the crop dormancy by winter (Klepeckas *et al.*, 2020). The grain weight of genotypes was relatively higher in 2017-18 with significant ($p<0.05$) differences for Pakistan-2013 (Fig. 3b). The weather difference has confirmed the relatively low temperature during the anthesis of the crop in 2017-18 at AP and AK with higher rainfall (Fig. 1), which adversely affected grain weight per spike. It is obvious that adverse climate at the crop critical stage, e.g. grain development, also affects grain weight and grain number (Sehgal *et al.*, 2018). Significant ($p<0.05$) differences were noticed in grain numbers for Pakistan-2013 and DN-84 in comparison with the rest of the genotypes (Fig. 3c).

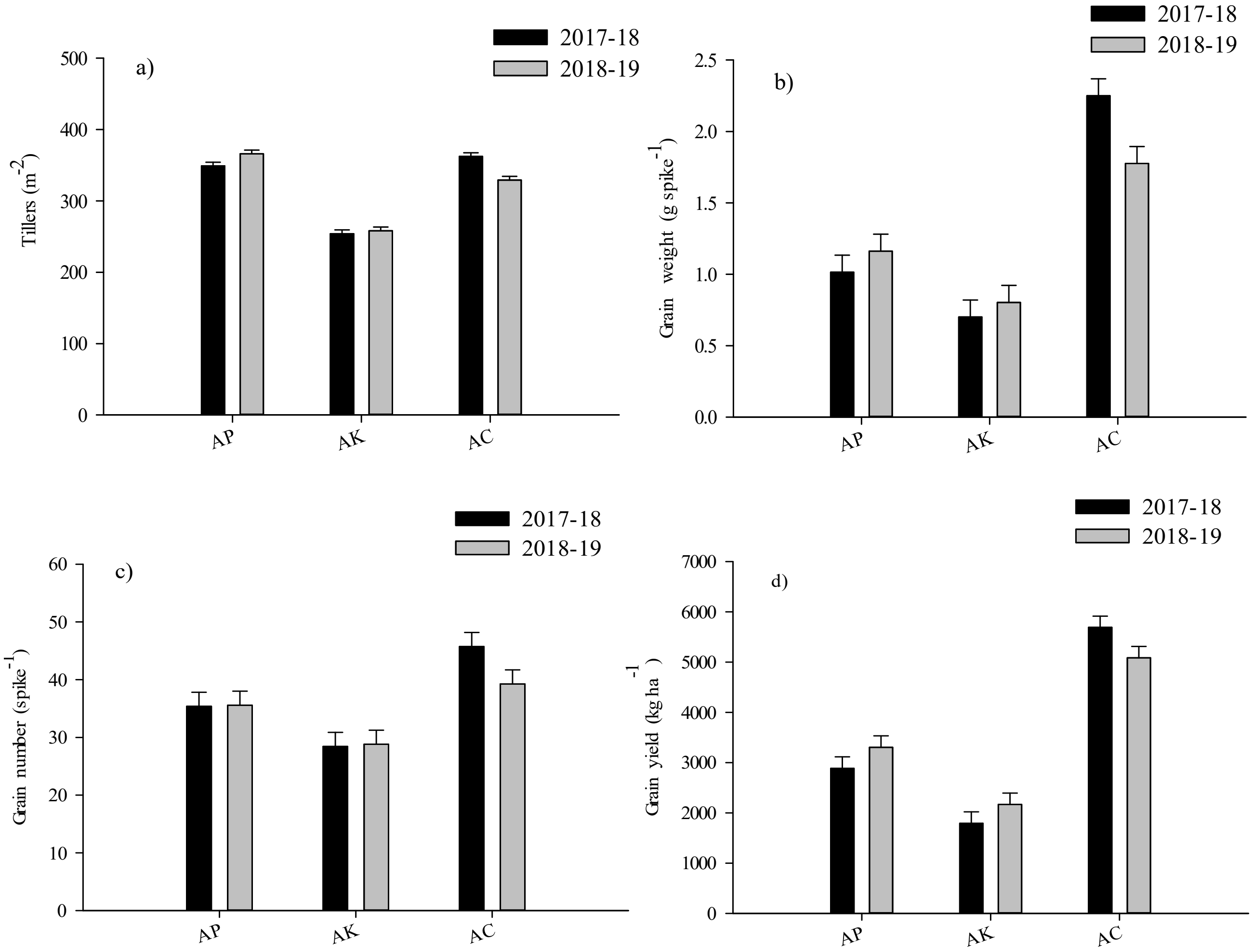


Fig. 2. Elevation x years treatment interaction for (a) tillers m^{-2} (b) grain weight (g spike^{-1}), (c) grain number spike^{-1} and (d) grain yield (kg ha^{-1}) of wheat genotypes in 2017-18 and 2018-19. LSD values are shown in bars.

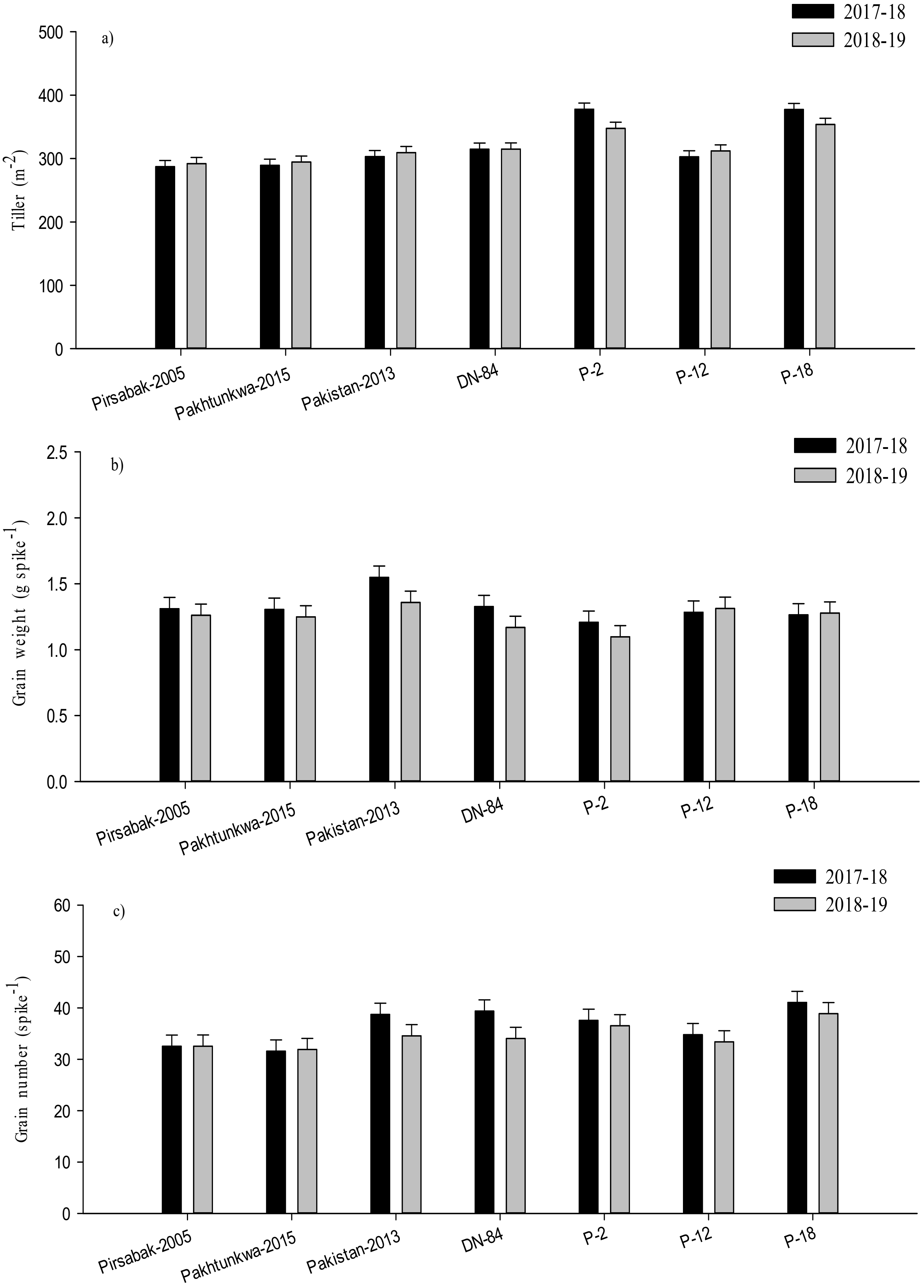
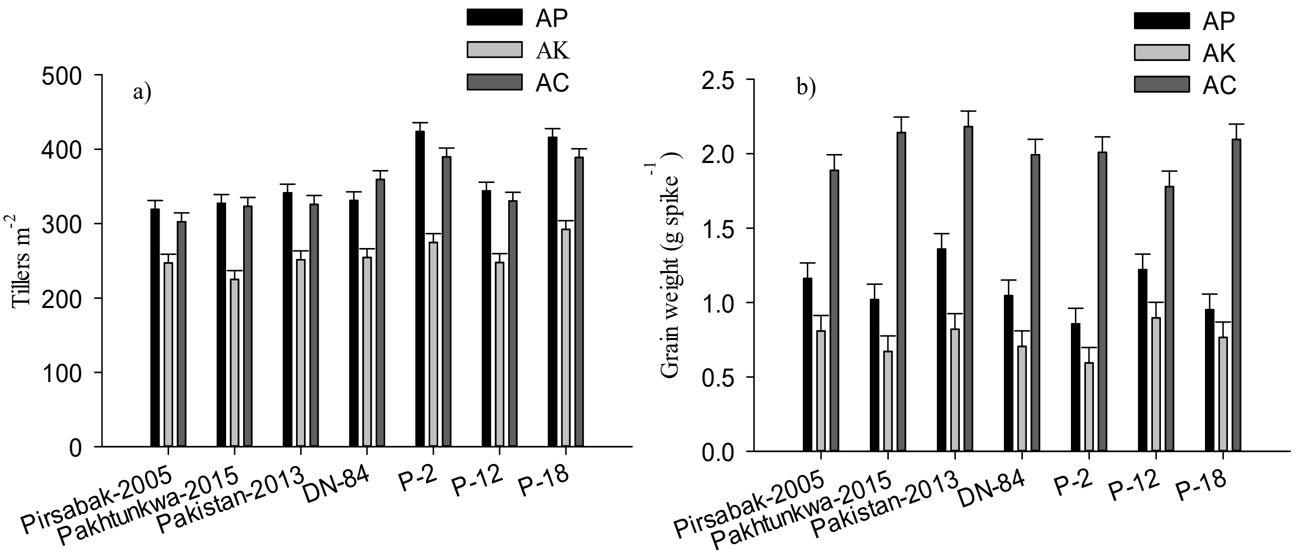


Fig. 3. Genotype x years treatment interaction for (a) tillers m^{-2} (b) grain weight (g spike^{-1}) and (c) grain number spike^{-1} of wheat genotypes in 2017-18 and 2018-19. LSD values are shown in bars.

The interaction G x E revealed significant changes in tiller number, grain weight, and number, and therefore the grain yield (Fig. 4). The highest number of tillers, more grains, and heavier weight resulted in the highest grain yield at AC over the rest of the two lower elevations. It may be due to the favorable growth conditions of the cooler climate at the highest altitudes that slowly contribute to the crop developmental stages that undergo during the crop life cycle with a slow increase in temperature. Contrary to this, the drought in AK resulted in lower grain number and weight, and therefore the grain yield despite the relatively cooler climate for crop growth and development than in AP. Drought alone affects crop growth more severely than high temperatures (Ullah *et al.*, 2019). Genotypes' response to elevation differed in different ways expressing changes in basic yield traits, i.e. tiller number, grain number and weight and hence final grain yield. Healthy traits expressed by a genotype in diversified elevations with or without water limitation are good for changing climates (Khaliq *et al.*, 2019). Performance of genotypes in diverse environments is possible if the genotype expresses healthy traits (Kamal *et al.*, 2019). Both climate and drought are limiting factors for plant growth, which cause differences in yield (Ullah *et al.*, 2019). Grain yield is of a genotype in three elevations if there are differences that limit its cultivation over the genotype that remained stable by changing altitude (Hussain *et al.*, 2021).

Treatment interaction (G x Y x E) revealed significant (p<0.05) differences in tiller number, grain weight and number (Fig. 5). As explained earlier, the majority of the two factors' interactions resulted in significant (p<0.05) changes in traits. Three ways interactions mimicked the same trend for tiller number, grain weight, and grain number for genotypes by altering altitudes. Temperature governs genotype growth. However, a change in radiation duration and intensity accelerates crop growth accordingly with an increase in elevation (Paudel *et al.*, 2021). A decrease in temperature by increasing elevation demands less water in the season but the same growth (Sehgal *et al.*, 2018). However, marked variations were observed in temperature at the three elevations, but drought remained dominant showing significant (p<0.05) changes in traits and yield. Among genotypes, only two lines differed in development from the rest five, but the primary traits (e.g. tiller number, grain number, and grain weight) differed within elevations and hence expressed changes in grain yield accordingly.



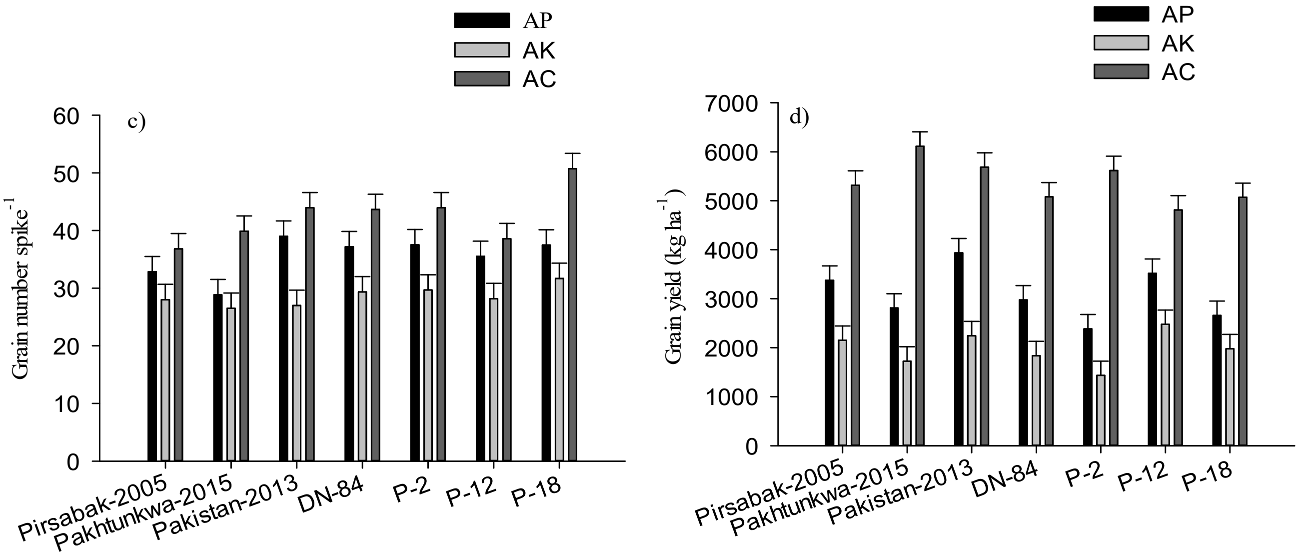


Fig. 4. Genotype x elevation treatment interaction for (a) tillers m^{-2} (b) grain weight (g spike^{-1}), (c) grain number spike^{-1} and (d) grain yield (kg ha^{-1}) of wheat genotypes in 2017-18 and 2018-19. LSD values are shown in bars.

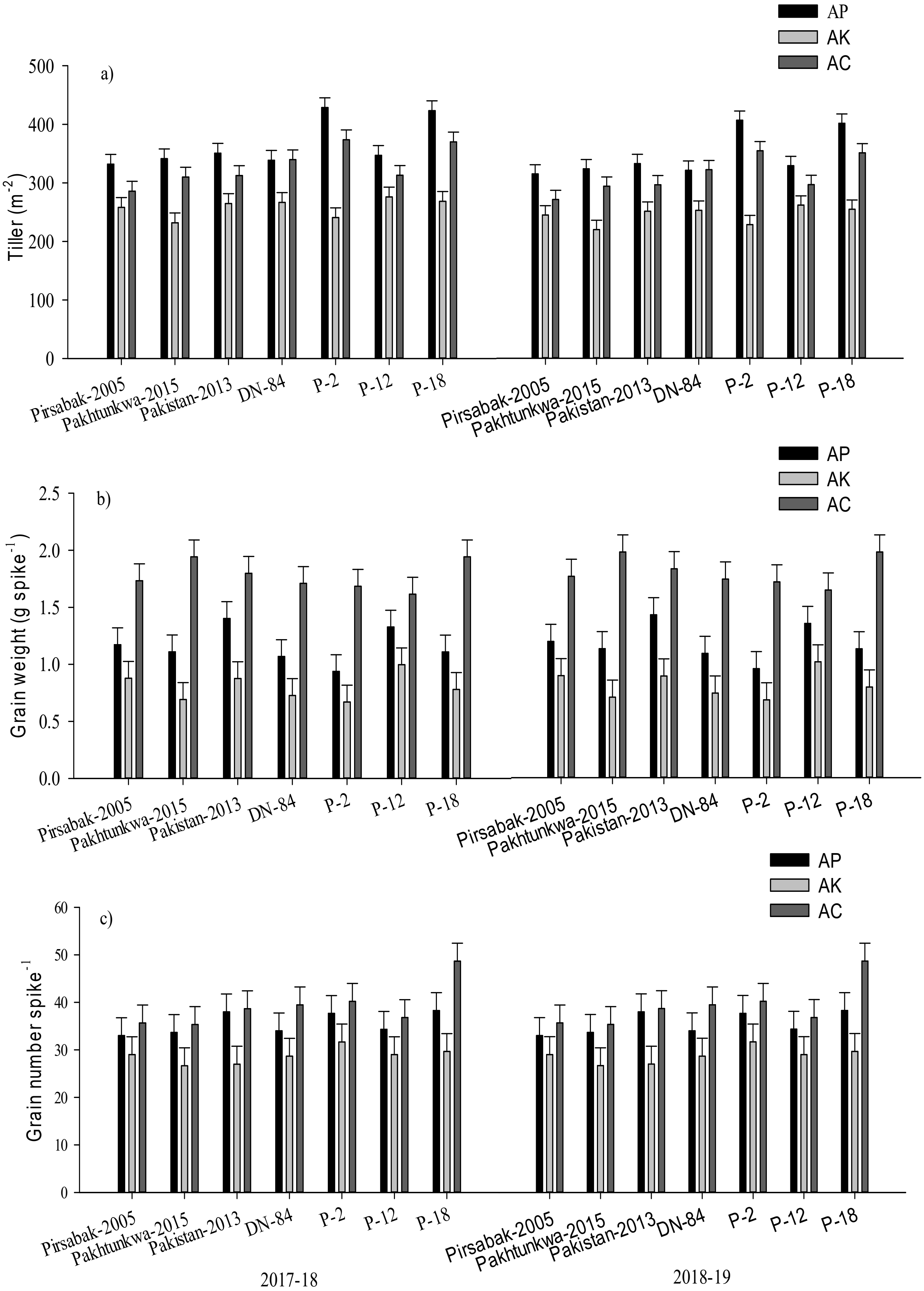


Fig. 5. Genotype x years x elevation treatment interaction for (a) tillers m^{-2} (b) grain weight (g spike^{-1}) and (c) grain number (spike^{-1}) of wheat genotypes in 2017-18 and 2018-19. LSD values are shown in bars.

Grain quality traits

Amylose, amylopectin, gluten and nitrogen (%): Grain quality traits, i.e. amylose, amylopectin, gluten and nitrogen content (%) were observed significant ($p<0.05$) with increasing elevation for wheat genotypes (Tables 3 & 4). Averaged over genotypes, two years mean data showed the highest (25.83%) amylose in AC, followed by AK (25.14%) and the lowest (22.51%) in AP. Similarly, the highest (77.50%) amylopectin was noticed in AP, followed by AK (74.90%) and the lowest (74.20%) in AC (Table 3). Wet gluten (%) was higher (28.5%) in AP, followed by AC (25.9%) and the lowest (23.4%) in AK. Higher (2.09%) grain N was noticed in AK and lower in AP (2.02%) with no change ($p<0.05$) from AC (Table 4). Amylose increased ($p<0.05$) with increasing elevation. This was due to stable assimilates accumulation in grain at low daily thermal accumulation rates. The change in amylose to amylopectin ratio is a temperature-related parameter of the developing grains. With the increasing temperature of the days, the rate of amylose in developing grains decreases (Impa *et al.*, 2021). Higher starch of wheat grain in AC was due to a prolonged grain growth period at high altitudes with stable grain fill duration (Kumar *et al.*, 2019). Changes in starch were noticed with a difference in the temperature of grain growth, which altered the ratio of amylose and amylopectin (Kato *et al.*, 2019). A quick temperature rise decreased starch and amylose (Sehgal *et al.*, 2018). The temperature difference between AK and AP differed but did not show a change due to the drought in AK. The drought effect is stronger than the temperature increase (Ali *et al.*, 2021). The low gluten content in grains of AP and AK in 2017-18 confirms the sowing date effect between years. Compared to amylose and amylopectin, gluten in grain was highly correlated with water. Literature confirmed that high temperature (+35°C) growth expressed lower levels of gluten by denaturing the protein. Gluten is a significant part of the protein (Khan *et al.*, 2021). Grain N was high in AK, followed by AP with no change in AC. The higher N in AK than in AC was due to drought, which affects grain weight and number, the dilution factor. The grain size was decreased in AK compared to AP, resulting in a higher N. It is obvious that growth at high temperatures expressed poor grain size and weight which resulted in high grain N (Kino *et al.*, 2020), Grain yield was reported to be higher ($p<0.05$) at 20/15°C but grain N at 28/23°C (Khan *et al.*, 2021), showing that high temperatures reflects higher grain N.

Averaged over elevations, genotypes differed ($p<0.05$) in amylose, amylopectin, gluten, and N-content. All parameters of quality differed in their nature of synthesis during growth (Ali *et al.*, 2021). Rates may differ within elevations due to changes in the growth rate under prevailing temperatures (Johansson *et al.*, 2020). Genotypes expressed variations ($p<0.05$) in grain weight and number due to assimilation rates from the source. Both leaf number and size affect grain weight. Moreover, spike weight and length also brought changes in grain weight (Klepeckas *et al.*, 2020. Genotypes also vary ($p<0.05$) for the timing of anthesis and grain fill duration to complete maturity by changing elevation. Pakistan-2013 and P-12 were relatively better ($p<0.05$) at expressing stable amylose contents (about 25%). Different genotypes have different starch accumulations (Kato *et al.*, 2019). Grain quality deteriorated if the crop experienced stress, i.e. drought or temperature(Johansson *et al.*, 2020) . High rains at grain development (AP) or drought (AK) at anthesis have confirmed poor grain quality with low amylose. Both temperature and water fluctuate grain amylose and amylopectin ratio (Kato *et al.*, 2019). Temperature increases the diameter of starch granules during grain filling, which differed within genotypes (Kato *et al.*, 2019). Higher wet gluten (30.8%) in Pirsabak-2005, followed by P-12 and Pakistan-2013, was due to grain sizes, weights, and rates of grain growth making differences in genotypes (Johansson *et al.*, 2020). Soil nutritional status and climate play an important role in plant growth. The N-content in grain was highest (2.20%) in Pakistan-2013 and Pirsabak-2005. Grain numbers per spike also differed within genotypes. Different grains per spike accumulated from the same source might differ in the accumulation of N (Kino *et al.*, 2020). Genotypes had different grain numbers, showing differences in N to 15%. Our results have expressed changes in grain number, and weight and N-content (Table 3).

The interactive effect of E x Y expressed higher amylose, amylopectin gluten and N-content in all three elevations between years (Fig. 6). Amylose was higher ($p<0.05$) in AP and AK in 2018-19 whereas amylopectin was reported low in AP and AK. Both temperatures and water played a role in grain quality. Differences in grain weight and number are associated with amylose and amylopectin ratios within different elevations, but changes in drought stress and temperature of elevations dominated the types. Gluten in grain is a protein that differed within elevations. Drought-over temperature (e.g. AK) adversely affects gluten. Compared to AC, the highest altitude expressed low gluten in AP. Total N in grains did not show any change with increasing elevation. Data confirmed the same grain N but changed amylose and amylopectin ratios by changing altitudes (Khan *et al.*, 2021).

Interaction (G x Y) expressed changes in amylopectin with higher amylose in 2018-19 due to a late sowing date (Fig. 7).

The interactive effect of G x E revealed changes in grain amylose, amylopectin, gluten, and N (Fig. 8). Grain quality is temperature and drought-sensitive. Literature has confirmed genotypes differ in assimilate production rates by sink size resulting in changes in grain size and numbers per spike (Ballesteros-Rodriguez *et al.*, 2019). The effect of genotypes in elevation gradients is established due to the expression of cytoplasm for growth (Harb *et al.*, 2020). Nonetheless, quantity performance is well documented, but quality traits are also needed to be observed. Assimilates production varies by genotypes for the synthesis of amylose, amylopectin, gluten, and N with changes under solar radiation intensity and duration, which differ within different elevations (Kumar *et al.*, 2019). Responses of genotypes also differed with elevation by changes in heat intensity as well as duration, affecting the amylose to amylopectin ratio, gluten, and N in grains.

Interaction Y x G x E expressed responses ($p<0.05$) in grain amylose to amylopectin (Fig. 9). The figure also confirmed effects between years for different elevations. We noticed an increase in amylose of a genotype reflects a similar decrease in amylopectin within an elevation. Genotypes with the stability of amylose vs. amylopectin with a ratio of 25:75 could be the better option to focus on its production with stable quality for different elevations.

Table 3. Grain amylose (%) and amylopectin content (%) of wheat genotypes planted at different elevations in 2017-18 and 2018-19.

Elevation (E)	Amylose (%)			Amylopectin (%)		
	2017-18	**2018-19**	**Mean**	**2017-18**	**2018-19**	**Mean**
AP	21.6	23.5	22.51 c	78.4	76.6	77.5 a
AK	22.6	27.7	25.14 b	77.4	72.3	74.9 b
AC	26.5	25.2	25.83 a	73.5	74.8	74.2 c
LSD (0.05) for E	0.51	1.25	0.6	0.5	1.3	0.6
Genotypes (G)						
Pirsabak-2005	21.5	26.6	24.0 c	78.6	73.4	76.0 b
Pakhtunkhwa-2015	23.9	25.9	24.9 ab	76.1	74.1	75.1 cd
Pakistan-2013	24.8	26.4	25.6 a	75.2	73.6	74.4 d
DN-84	23.8	25.1	24.4 bc	76.2	74.9	75.6 bc
P-2	23.8	24.8	24.3 bc	76.2	75.2	75.7 bc
P-12	23.5	26.8	25.2 a	76.5	73.2	74.8 d
P-18	23.6	22.5	23.1 d	76.4	77.5	77.0 a
LSD (0.05) for G	0.77	1.14	0.68	0.77	1.14	0.68
Year (Y) mean	23.6	25.4	***	76. 5	74.6	***
Significance level (p<0.05) for treatment interaction						
E x Y	-	-	**	-	-	**
G x Y	-	-	**	-	-	**
G x E	**	**	**	**	**	**
Y x G x E	-	-	**	-	-	**

Statistically similar means within a category of treatments are represented by the same letters using the least significant difference (LSD) test (*p*<0.05); Significant level * (*p*<0.05), ** (*p*<0.01) and NS = Non-significant

Table 4. Grain wet gluten (%) and nitrogen (%) of wheat genotypes planted at different elevations in 2017-18 and 2018-19.

Elevation (E)	Wet gluten content (%)			Nitrogen content (%)		
	2017-18	**2018-19**	**Mean**	**2017-18**	**2018-19**	**Mean**
AP	27.9	29.1	28.5 a	2.0	2.0	2.02 b
AK	21.1	25.7	23.4c	2.1	2.1	2.09 a
AC	26.6	25.2	25.9 b	2.1	2.0	2.02 b
LSD (0.05) for E	1.0	1.2	0.7	0.1	0.1	0.04
Genotypes (G)						
Pirsabak-2005	30.1	31.6	30.8 a	2.1	2.1	2.09 b
Pakhtunkhwa-2015	23.5	24.9	24.2 d	1.9	2.0	1.94 d
Pakistan-2013	26.1	27.7	26.9 b	2.1	2.1	2.12 b
DN-84	24.5	25.8	25.2 c	1.9	2.0	1.95 cd
P-2	20.4	21.4	20.9 e	2.0	2.0	2.01 cd
P-12	27.1	28.5	27.8 b	2.2	2.2	2.20 a
P-18	24.7	26.8	25.7 c	2.0	2.0	2.01 c
LSD (0.05) for G	1.2	1.5	0.9	0.1	0.1	0.07
Year (Y) mean	25.2	26.7	***	2.05	2.05	NS
Significance level (*p*<0.05) for treatment interaction						
E x Y	-	-	**	-	-	*
G x Y	-	-	NS	-	-	NS
G x E	**	**	**	**	**	**
Y x G x E	-	-	NS	-	-	NS

Statistically similar means within a category of treatments are represented by the same letters using the least significant difference (LSD) test (*p*<0.05); Significant level * (p<0.05), ** (p<0.01) and NS = Non-significant

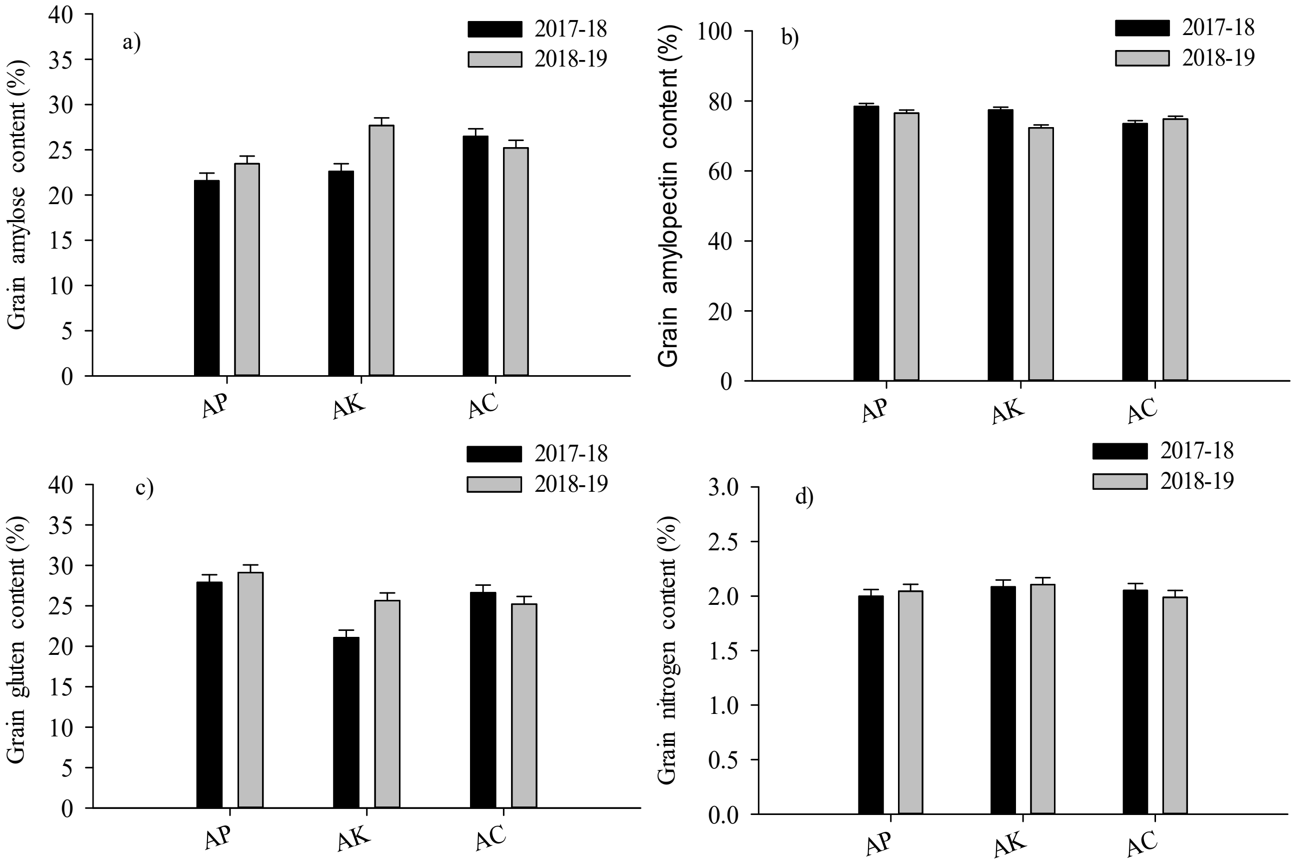


Fig. 6. Elevation x years treatment interaction for grain (a) amylose content (%), (b) amylopectin content (%), (c) gluten content (%) and (d) nitrogen content (%) of wheat genotypes in 2017-18 and 2018-19. LSD values are shown in bars.

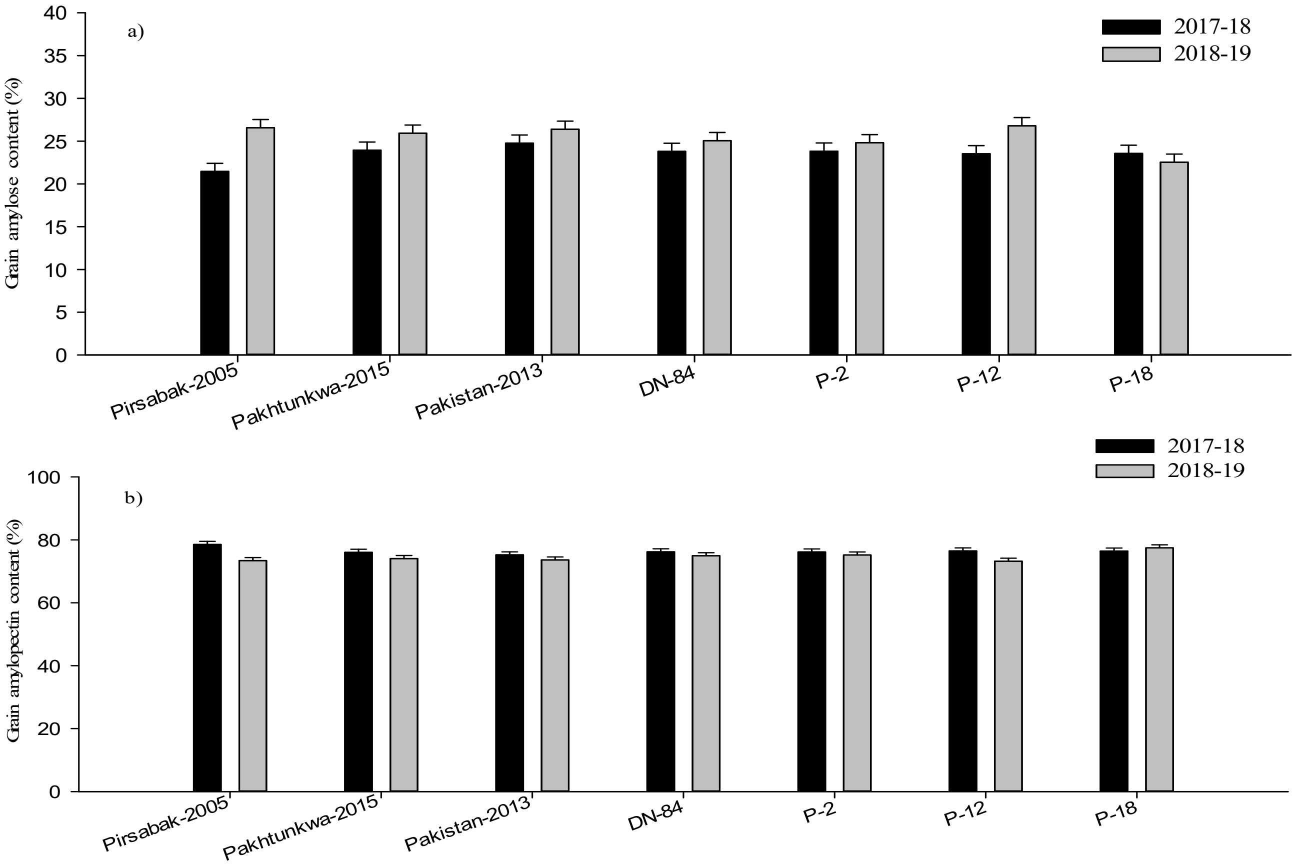


Fig. 7. Genotype x years treatment interaction for grain (a) amylose content (%) and (b) amylopectin content (%) of wheat genotypes in 2017-18 and 2018-19. LSD values are shown in bars.

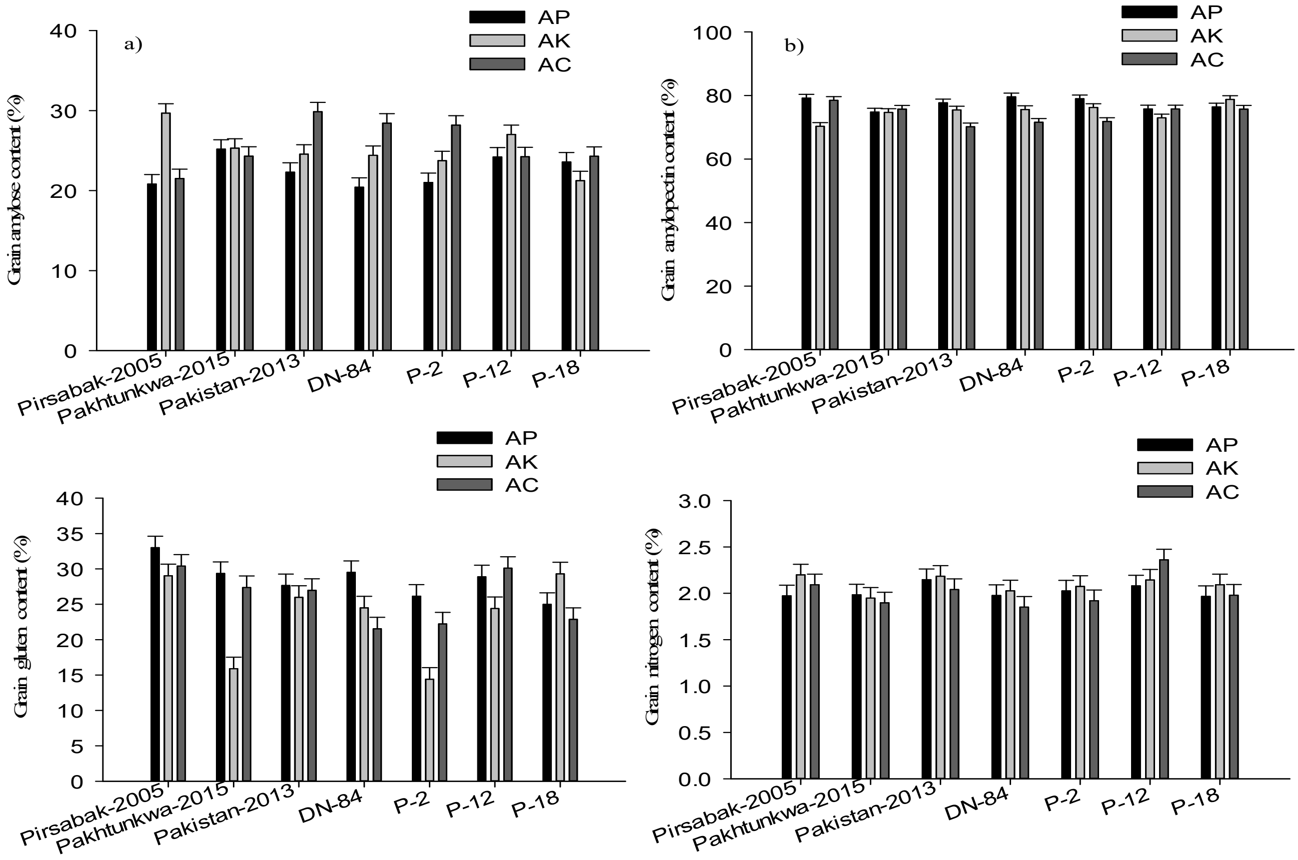


Fig. 8. Genotype x elevation treatment interaction for grain (a) amylose content (%), (b) amylopectin content (%) (c) gluten content (%) and (d) nitrogen content (%) of wheat genotypes in 2017-18 and 2018-19. LSD values are shown in bars.

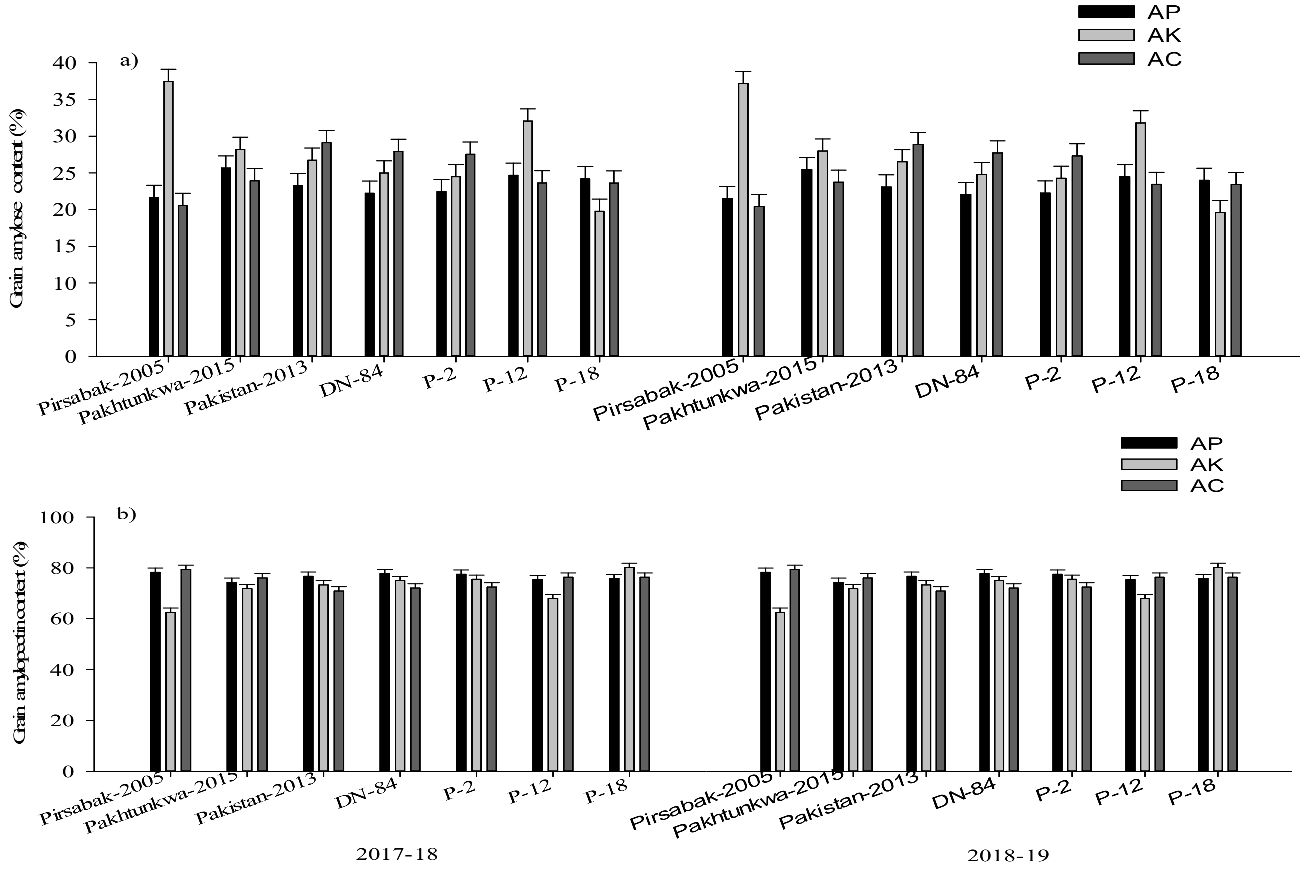


Fig. 9. Years x genotype x elevation treatment interaction for grain (a) amylose content (%) and (b) amylopectin content (%) of wheat genotypes in 2017-18 and 2018-19. LSD values are shown in bars.

Conclusions

No compromise on yield increase for the growing population to overcome food security. However, quality should not be over-looked. Genotypes with stable performance from low to high elevations might have to take in consideration for stability in amylose to amylopectin ratio. A stable ratio of amylose to amylopectin with optimum production at both low and high altitudes ensures food security. Nonetheless, gluten is an important quality trait for backing and Pakhtunkhwa-2015 including advanced lines (P-12 and P-18) guarantees this trait for future breeding wheat to cope with climate change effect on the wheat crop.

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