

## Nitrogen application rate and timing management for improved grain quality parameters of wheat crop

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Nitrogen use efficiency under flood irrigation system is generally low (30%) in field crops, which is one of the fundamental factors of high production cost in the developing countries. Optimum rate and timing of N-application is otherwise important to harvest good quality grain for backing in the recent climate change scenario. Optimum N-rate (NAR) corresponds with the application timing (NAT) has resulted in good quality grains. Aim of the study was to focus on spring wheat grain quality and N use efficiency (NUE) with NAR {i.e., 0, 100, 120, 140 and 160 kg ha<sup>-1</sup>} and NAT (i.e., 100% at sowing (NAR<sub>1</sub>), 50% at sowing and 50% at tillering (NAT<sub>2</sub>), 25% at sowing, 50% at tillering and 25% at booting (NAT<sub>3</sub>) and 25% at sowing, 25% at tillering and 50% at booting (NAT<sub>4</sub>)). Treatment impacts were investigated focusing grain yield, grain-N, and quality parameters (i.e., crude protein, gluten, amylose and amylopectin). Experiment was a randomized complete block, in three replications, conducted at Agronomy Res. Farm of the University of Agric. Peshawar in 2016-17 and repeated in 2017-18. Results showed the highest NUE in 100 kg N ha<sup>-1</sup>, followed by a decreasing rate (p<0.05) for every next N-increment. While averaged on N-rates, the highest NUE observed in NAT<sub>3</sub> which did not differ from NAT<sub>4</sub> but decreased (p<0.05) for treatment NAT<sub>2</sub> with lowest for the NAT<sub>1</sub>. Pakhtunkhuwa-2015 showed higher NUE among the varieties. Grain-N, grain yield, gluten and amylose did not differ with NAR 140 and 160 kg ha<sup>-1</sup> as well as for the NAT<sub>3</sub> and NAT<sub>4</sub> but decreased for NAT<sub>2</sub> and the lowest was noted for NAT<sub>1</sub>. The N-content of wheat grain was highest in Pakhtunkhuwa-2015, followed by Pirsabak-2015 and the lowest in DN-84. Nonetheless, grain amylopectin showed a reduction with increasing NAR and/or split N-applications from one to two and/or three doses. Cultivars did not show any changes in the amylopectin. It is concluded that in recent climate changes where flood irrigation system is practiced, three N-splits (NAT<sub>3</sub> or NAT<sub>4</sub>) resulted higher quality grains with 140 kg N ha<sup>-1</sup> to wheat crop.

**Keywords:** Nitrogen rate, nitrogen timing, N-use efficiency, yield traits and grain yield, wheat varieties.

### INTRODUCTION

Wheat (*Triticum aestivum* L.), being an important cereal crop grown throughout the globe, is a major source of staple food and feed for living being. It is one of the most important crops in central Asia and Pakistan, used as a staple food to meet nutritional requirements with higher calories and protein of an average human diet (Adhikari *et al.*, 2017). On average, it provides 22% protein and 20% calories (Pfeifer *et al.*, 2014) and is extensively used in food due to gluten properties (Elli *et al.*, 2015). Gluten is gliadin and glutenin, and its viscosity linked with the gliadin (Barak *et al.*, 2013). Agronomic and genetics techniques are adopted to unravel the wheat flour and backing quality (Sher, 2019). Particularly, the N fertilization regulates rate and timing to harvest better quality grains for backing.

Nitrogen (N) is an important component of chlorophyll, various hormones, nucleotides, vitamins and enzymes supported vegetative growth of plants (Delloye *et al.*, 2018). Nitrogen is a major constituent of amino acids, building blocks of proteins, essential for grains formation (Duan *et al.*, 2020) and to harvest the optimum yield (Preece *et al.*, 2017). Balanced N application rate and timings to the crop is a key for successful production (Aczel, 2019). Nitrogen is one of the most important inorganic fertilizer, consumed the maximum than any other nutrients and a major limiting factor of plant growth in term of yield and quality of the finish product (Agegnehu *et al.*, 2016). Nitrogen plays a fundamental role in improving wheat grain quality (Medford *et al.*, 2017). Initially, single N application was recommended for the crop growth and development, followed by two splits

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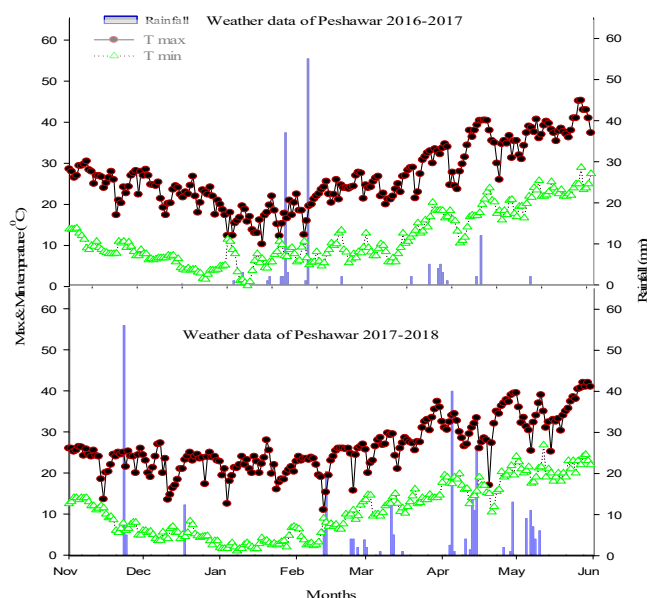
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with good responses (Adeyemi *et al.*, 2020). The recent changes in seasonal climate have also changed the pattern of rainfall and durations, which is expected to be continued till 2040 in Pakistan (Asim *et al.*, 2013; Hanif and Ali, 2014). During these changes, wheat crop will adversely be affected in rainfed areas at sowing time and higher infestation of disease and insect attack in irrigated land closer to the anthesis stage of growth and development. Nitrogen under dryland and/or excessive rainfalls will be lost by leaching and volatilization in soil (Jones *et al.*, 2013; Ni *et al.*, 2014). To avoid such N-loss, it is important to focus on application timing with proper rates subjected to the plant growth and development for optimum utilization. This would not only decrease the input costs of production but also increase the NUE with better quality grain (Cormier *et al.*, 2016; Herrera *et al.*, 2016). Moreover, the N application rate can optimize growth and grain N content, protein and baking quality (Ooro *et al.*, 2011; Xue *et al.*, 2019) as quality relates to gliadins and glutenin strengthening viscosity and extensibility (Cappelli *et al.*, 2020). Split N-application has strengthened flour loaf volume (Xue *et al.*, 2019). Protein content of wheat grains depends upon the N-application rate, timing, soil fertility, cultivar and climatic condition (Geleta *et al.*, 2002). For better grain yield, crude protein and nitrogen use efficiency (NUE), split N-application during growth is more effective (Mohammed *et al.*, 2013). Nitrogen fertilization plays important role for yield and quality and can easily be adjusted with application of optimum N rate corresponds with the crop growth (Zoerb *et al.*, 2010). Nitrogen application at different stages of the plant (e.g., sowing, tillering and booting) has increased N-uptake, NUE, starch and protein content of grains (Da-Moraes *et al.*, 2013). To produce higher grain yield and with better quality, N should be applied with corresponds to growth stages of the crop (Leghari *et al.*, 2016). On the other hand, increasing NUE, proper N-rate and application timings, as fertilizer, is essential for good crop husbandry and grain quality (Hirel *et al.*, 2011). For good baking quality, grains must have enough protein content, which can be sustained to be made available in the soil at the time of grain development (Blumenthal *et al.*, 2008). The recent climate shift in Pakistan has shown an increase in rainfall intensity and duration when wheat enters in reproductive stages of the growth (Hanif and Ali, 2014). Focusing N-application timing and rates for wheat as staple food crop is of high significant to address grain yield and quality (Beleta *et al.*, 2018).

This study was, therefore, conducted to elucidate multiple split N application timing and N-application rates addressing the spring wheat yield, grain N-content, gluten, amylose and amylopectin, which adds in loaf volume, baking quality and kneading rheology of the crop. Nitrogen is highly mobile in soil and is applied in greater quality than any other nutrient in two splits and recent climate change has shown higher rainfalls in wheat reproductive stage.

## MATERIALS AND METHODS

**Experimental station:** Two years field experiments were conducted at Agronomy Research Farm, the University of Agriculture Peshawar-Pakistan located at (34.01°N, 71.35°E, altitude 350m and 1600 km North of the Indian ocean). Climate of the Peshawar is semi-arid, relatively hot summer with mild cool and relatively shorter winter. The mean maximum and minimum temperatures of summer are 25-40 °C and winter 4-18°C. Soil is, relatively silt loam, alkaline and low in organic matter. Weather data such as maximum, minimum temperatures (°C) and rainfall (mm) of the crop growth period are shown in Fig. 1.



**Figure 1. Daily rainfall (vertical bar), mean maximum and minimum temperature (line) of experimental location for the crop growth seasons (2016-17 and 2017-18).**

**Experiment details:** To plant the crop, seedbed was prepared as recommended for wheat in the area. Field after maize harvesting was plowed with cultivator and subsequently planked. The experiment was laid out in randomized complete block design with split plots arrangements using three replications. Pre-basic seeds of wheat (cv. Pakhtunkhwa-2015 which is recommended for both rain-fed and irrigated area, DN-84 approved for rain-fed and Pirsabak-2015 recommended as irrigated) were planted on November 21, 2016, and November 29, 2017, using same seed rate 120 kg ha<sup>-1</sup> for all varieties. Planting was made manually using a hand-driven hoe. Phosphorus (90 kg ha<sup>-1</sup>) and potassium (60 kg ha<sup>-1</sup>) were applied as recommend at the time of the seed-bed preparation using single super phosphate (18%) and Muriate of potash (60%). Nitrogen was applied in different rates (i.e., 0, 100, 120, 140 and 160 kg ha<sup>-1</sup>) on different stages

i.e. at sowing time 0 days after sowing (DAS), tillering (70 DAS) and at the crop booting stages (100 DAS) using urea source (N-46%). The different N-timings were NAT<sub>1</sub> (Single application at seedbed preparation), NAT<sub>2</sub> (Two splits 50% at sowing and 50% at tillering), NAT<sub>3</sub> (three splits 25% at sowing, 50% at tillering and 25% at booting) and NAT<sub>4</sub> (three splits 25% at sowing, 25% at tillering and 50% at booting) of the suggested rates. Field was irrigated uniformly at tillering, booting and grain filling stages as per crop water need. All other practices were kept uniform for the experimental units. Herbicide (Affinity 50WP, 2.0) was used for weeds control at tillering stage once as post emergence spray.

**Grain yield:** Grain yield (g m<sup>-2</sup>) was recorded by harvesting four central rows in an experimental unit, bundled and dried for 10 days in field. Dried bundles were threshed on a mini lab wheat thresher. Grains were collected, weighed and adjusted for grains moisture content (16% grain moisture) to estimate the grain yield.

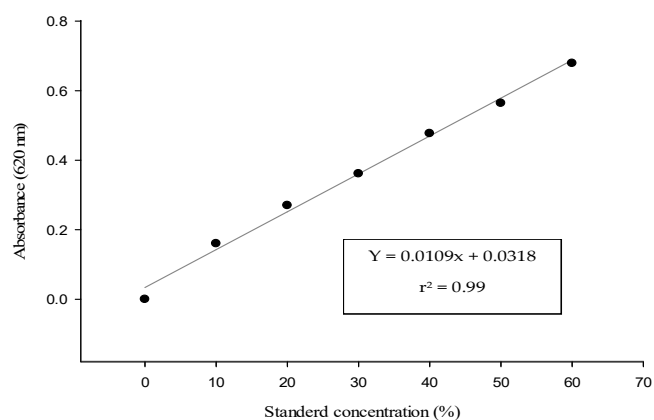
**Nitrogen content:** Grain N-content was determined in composite samples (%) taken from all experimental units after threshing the crop. Sample was dried, ground (Cyclone Mill Twister), conserved and analyzed for grain N-content. Nitrogen was determined by Kjeldahl method (Kozin *et al.*, 2019). Briefly, grain and straw samples were weighed (0.2 g), added with 1.3 g of digestion mixture (CuSO<sub>4</sub> 10 g + K<sub>2</sub>SO<sub>4</sub> 100 g and selenium metal powder 1 g) included 3 ml of concentrated H<sub>2</sub>SO<sub>4</sub> in a digestion tube and assembled. Contents of digestion tube was heated continuously till solution became clear greenish, filtered, volume made 100 ml and transferred to a Kjeldhal apparatus. A 20 ml sample was added with 4 ml NaOH solution and heated for distillation, distillate was collected in a flask containing 5 ml of already made boric acid mixed indicator and heated till yellowish color appeared. Distillate was titrated with 0.005 N HCl. Readings for sample as well as blank was recorded. Total N-content was derived as mean value of grain of the treatments. Respective N-content multiplied with a factor (6.25) for crude protein determination.

**N use efficiency:** Nitrogen use efficiency (NUE) was derived as per procedure explained by Fageria and Nasciente (2014). Briefly explained as the ratio of the grain weight harvested from a plot treated with a N-rate less yield of the control plot divided by the given N-rate to that plot. The reading of NUE was expressed in percentages.

**Seed gluten:** Hand wash method was used for seed gluten (%) determination (Sayaslan *et al.*, 2006). Grains were ground in a mini-lab grinding mill (Cyclone Mill Twister, 50/60 Hz). A 25g sample collected, mixed with 15ml of water to form wet dough. Dough was allowed to stand for an hour. All starch and soluble matter were removed by pressing dough in water flowing in a plastic dish. Dough-ball was placed in a tarred, flat-bottom dish and weighted as wet gluten. Dough-ball was dried at 100°C in an oven for dry gluten content. Gluten (%)

was determined by taking ratio of wet gluten over the sample weight and expressed value in percentages.

**Amylose content:** Amylase (%) was determined by iodine calorimetric method (Nakamura *et al.*, 2020). Grains were grounded and passed through a 100 mm mesh. Sample of 100 mg was taken in triplicate in 100 ml flask, added with 1.0 ml (95%) ethanol and 9 ml NaOH (IM) solution. Contents of flasks were boiled in a water-bath for 10 minutes to gelatinize the starch. Boiled samples were allowed to rest for a while, volume was made to 100 ml by adding distilled water and store at the room temperature (25°C) for 23h. A blank was also prepared in addition to sample for correction. Solution in flask was mixed, a 5.0 ml sample was pipetted into another 100 ml flask, 1 ml of glacial acetic acid (IM) and 2 ml iodine (I<sub>2</sub> is insoluble in water, 2 g potassium iodide in 900 ml of distilled water was added in 1g iodine crystals), adjusted volume 900 ml with distilled water. Sample was mixed and rest for 20 minutes, which developed a dark blue color. Spectrophotometer was calibrated to run standard at 620 nm spectrum along with all samples. Value of absorbance was converted to amylose using standard calibration curve developed from the pure potato amylose (Fig. 2).



**Figure 2. Amylose standard curve developed for amylose in wheat grains. Solid dot shows actual value against the regression.**

**Amylose curve:** Pure amylose 12.5 mg (0.0125 g) and amylopectin 12.5 mg were taken in separate tube, added with 5 ml of 45% perchloric acid and dissolved. Bring final volume to 50 ml with distilled water. A 6.25 ml amylose stock from 50 ml, added with 18.75 ml ultra-pure water to make 6.25 mg/ml amylose solution, repeated the same procedure for amylopectin solution. Using this stock solution, standard solution of amylose and amylopectin was prepared in percent (0, 10, 20, 30, 40, 50 and 60%) to a final volume of 5 ml to create standard curve. For example, to prepare 0% amylose / 100% amylopectin standard, pipette 5 ml amylopectin solution. A 10% amylose / 90% amylopectin standard combined 0.5 ml amylose and 4.5 ml amylopectin solution. A 20% amylose / 80% amylopectin standard combine 1.0 ml

amylose and 4.0 ml of amylopectin solutions the same ratio (0.5) were used for 10% intervals. Transfer 40 µl of a standard mixture to a micro-titer plate. Add 50 µl of iodine solution (2 g KI + 1g I<sub>2</sub> in 900 ml of ultra-pure water) and mix each sample including blank (perchloric acid) by pipetting. Read absorbance at 620 nm immediately and calculate amylose ratio for a concentration. A standard calibration curve was developed using different concentrations and their absorbance (Fig. 2).

**Statistical analysis:** Data were statistically analyzed using analysis of variance techniques appropriate for randomized complete block design with split plot arrangements (Izenman, 2008; Box, Jones, 1992) for a single year and two years averages. R-3.5.3 software was used for analyzing the data. Means were separated using the least significance difference (LSD) test at a 5% level of significance.

## RESULTS

**Grain N-content and grain yield:** Grain N-content (GNC) as

influenced by nitrogen application rate (NAR, nitrogen application timing (NAT) and varieties revealed a significant change (Table 1). Averaged across NAT and varieties, GNC affected ( $p < 0.05$ ) with highest for 140 kg ha<sup>-1</sup> which did not differ ( $p < 0.05$ ) from 160 kg ha<sup>-1</sup>. The NAR 100 kg ha<sup>-1</sup> also did not differ ( $p < 0.05$ ) from 120 kg ha<sup>-1</sup> with lowest reading for control (0 kg ha<sup>-1</sup>). Regarding NAT, three splits (i.e., NAT<sub>3</sub>) resulted in high GNC, which was statistically similar with (NAT<sub>4</sub>). Two splits (NAT<sub>2</sub>) were lower for GNC and lowest GNC under NAT<sub>1</sub>. While averaged across NAR and NAT, varieties showed differences in GNC with highest in Pakhtunkhwa-2015, followed by Pirsabak-2015 and lowest in DN-84. Among interactions, NAR x V was significant (Fig. 3).

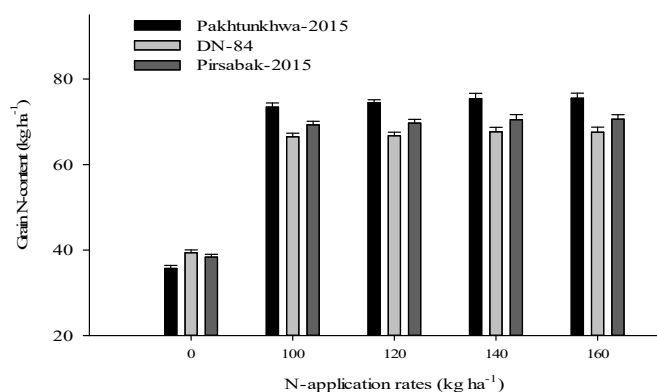
GNC showed a visible increment for all varieties when NAR increased from 0 to 100 kg ha<sup>-1</sup>, thereafter GNC leveled off in all three varieties by increasing NAR from 100 to 160 kg ha<sup>-1</sup>. Nevertheless, Pakhtunkhwa-2015 was better than Pirsabak-2015, which was followed by DN-84 at any given N-rate from 100 to 160 kg ha<sup>-1</sup>.

**Table 1. Grain and tissue nitrogen (kg ha<sup>-1</sup>) of wheat as influenced by N-application rates (NAR) and N-application timings (NAT).**

N-application rates (kg ha <sup>-1</sup> ) NAR	Grain nitrogen (kg ha <sup>-1</sup> )			Grain yield (g m <sup>-2</sup> )		
	2016-17	2017-18	Means	2016-17	2017-18	Means
0	37.9	37.8	37.8 c	230.4	226.5	228.5 d
100	76.9	63.2	70.1 b	357.8	299.5	328.6 c
120	77.2	63.5	70.3 b	366.5	301.4	334.0 b
140	78.0	64.4	71.2 a	371.6	306.9	339.3 a
160	78.2	64.3	71.3 a	372.0	306.9	339.5 a
LSD (0.05)			0.5			3.9
Nitrogen Timings (NAT)						
NAT <sub>1</sub>	68.7	58.0	63.4 c	332.7	297.2	315.0 c
NAT <sub>2</sub>	69.7	58.4	64.1 b	338.3	299.5	318.9 b
NAT <sub>3</sub>	70.2	59.0	64.6 a	344.5	304.7	324.6 a
NAT <sub>4</sub>	69.9	59.0	64.5 ab	343.1	304.7	323.9 a
LSD (0.05)			0.4			3.5
Varieties						
Pakhtunkhwa-2015	73.8	60.5	67.1 a	351.3	301.5	326.4 a
DN-84	66.0	57.2	61.6 c	336.9	275.7	306.3 c
Pirsabak-2015	69.1	58.3	63.7 b	330.8	287.6	309.2 b
LSD (0.05)			0.5			3.1
Years means	69.6	58.6	**	339.7	288.3	**
Interactions						
NAR x NAT	ns	ns	ns	ns	ns	ns
NAR x V	**	ns	**	**	ns	**
NAT x V	ns	ns	ns	ns	ns	ns
NAR x NAT x V	*	ns	ns	*	ns	ns

NAT<sub>1</sub> = 100% at sowing; NAT<sub>2</sub> = 50% at sowing (0 DAS) & 50% at tillering (70 DAS); NAT<sub>3</sub> = 25% at sowing (0 DAS), 50% at tillering (70 DAS) & 25% at booting stage (100 DAS); NAT<sub>4</sub> = 25% at sowing (0 DAS), 25% at tillering (70 DAS) & 50% at booting stage (100 DAS)

Means followed by different letter within a category of a treatments are statistically different from each other using least significant difference (LSD) test ( $p < 0.05$ ).



**Figure 3.** Grain nitrogen (kg ha<sup>-1</sup>) of treatment interactions [N application rates (NAR) x wheat variety].

Grain yield (g m<sup>-2</sup>) as influenced by NAR, NAT and cultivars showed significant responses to the given treatments (Table 1). While averaged across NAT and cultivars, the maximum grain yield was reported under NAR 140 kg ha<sup>-1</sup> which did not differ from treatment NAR 160 kg ha<sup>-1</sup>,

followed by NAR 120 and 100 kg ha<sup>-1</sup>. The minimum grain yield was observed in control treatment (0 kg ha<sup>-1</sup>). Irrespective of the NAR and cultivars, grain yield showed changes ( $p < 0.05$ ) with NAT with maximum yield in three splits (NAT<sub>3</sub> or NAT<sub>4</sub>) with either splits-application ratios, followed by two splits (NAT<sub>2</sub>) and the minimum for single application (NAT<sub>1</sub>). Among the cultivars, Pakhtunkhwa-2015 showed higher grain yield, followed by Pirsabak-2015 and the lower for DN-84. Among treatment interaction (NAR × V) was significant for grain yield almost same response for grain yield, variety Pakhtunkhwa-15 and Pirsabak-15 showed prominent increase as compared to DN-84 at any NAR (Fig. 4).

**Nitrogen use efficiency (%):** Nitrogen use efficiency (NUE) as influenced by NAR, NAT and wheat was significant (Table 2). Averaged across NAT and varieties, increasing NAR from 100 to 160 kg ha<sup>-1</sup> has showed a significant decrease with each increment. Obviously, the maximum NUE was recorded in the 100 kg N ha<sup>-1</sup>, which decreased ( $p < 0.05$ ) for 120 and 140 kg N ha<sup>-1</sup>. The lowest NUE was observed at 160 kg ha<sup>-1</sup>. Means across NAR and varieties, NAT did differ in NUE with highest for three splits (i.e. NAT<sub>3</sub>), followed by

**Table 2.** Nitrogen use efficiency (%) and crude protein (%) of wheat as influenced by N-application rates (NAR) and N-application timings (NAT).

N-application rates (kg ha <sup>-1</sup> ) NAR	Nitrogen use efficiency (%)			Crude protein (%)		
	2016-17	2017-18	Means	2016-17	2017-18	Means
0	-	-	-	9.8	9.5	9.6 c
100	35.8	36.6	36.2 a	12.3	11.9	12.1 b
120	30.5	30.3	30.4 b	12.6	11.8	12.2 b
140	26.5	27.2	26.8 c	13.0	12.1	12.6 a
160	24.6	24.6	23.9 d	12.7	12.9	12.8 a
LSD (0.05)			0.5			0.3
Nitrogen Timings (NAT)						
NAT <sub>1</sub>	28.3	27.6	28.0 c	11.7	11.2	11.4 b
NAT <sub>2</sub>	28.9	29.7	29.3 b	11.7	11.2	11.4 b
NAT <sub>3</sub>	29.5	30.7	30.1 a	12.3	11.5	11.9 a
NAT <sub>4</sub>	29.4	30.6	30.0 a	12.7	11.1	11.9 a
LSD (0.05)			0.50			0.2
Varieties						
Pakhtunkhwa-2015	30.1	29.5	29.8 a	11.7	11.2	11.5 c
DN-84	28.7	29.3	29.0 b	12.1	11.6	11.8 b
Pirsabak-2015	28.2	30.2	29.2 b	12.5	12.1	12.3 a
LSD (0.05)			0.3			0.2
Years means	29.0	29.7	Ns	12.1	11.6	Ns
Interactions						
NAR x NAT	Ns	ns	**	Ns	ns	**
NAR x V	Ns	ns	Ns	**	ns	Ns
NAT x V	Ns	ns	Ns	Ns	ns	*
NAR x NAT x V	Ns	ns	Ns	*	ns	Ns

NAT<sub>1</sub> = 100% at sowing; NAT<sub>2</sub> = 50% at sowing (0 DAS) & 50% at tillering (70 DAS); NAT<sub>3</sub> = 25% at sowing (0 DAS), 50% at tillering (70 DAS) & 25% at booting stage (100 DAS); NAT<sub>4</sub> = 25% at sowing (0 DAS), 25% at tillering (70 DAS) & 50% at booting stage (100 DAS). Means followed by different letter within a category of a treatments are statistically different from each other using least significant difference (LSD) test ( $p < 0.05$ ).

NAT<sub>4</sub> and two splits (i.e. NAT<sub>2</sub>) with lowest for single application. On averaged across NAR and NAT, varieties found significant for NUE with higher for Pakhtunkhwa-2015, followed by DN-84, which did not differ from Pirsabak-2015. Among interaction, only NAR x NAT was significant for NUE (Fig. 5). Better NUE observed under three splits (i.e. NAT<sub>3</sub> and NAT<sub>4</sub>), followed by two splits (NAT<sub>2</sub>) and lowest under NAT<sub>1</sub> with decreasing trend for increased NAR.

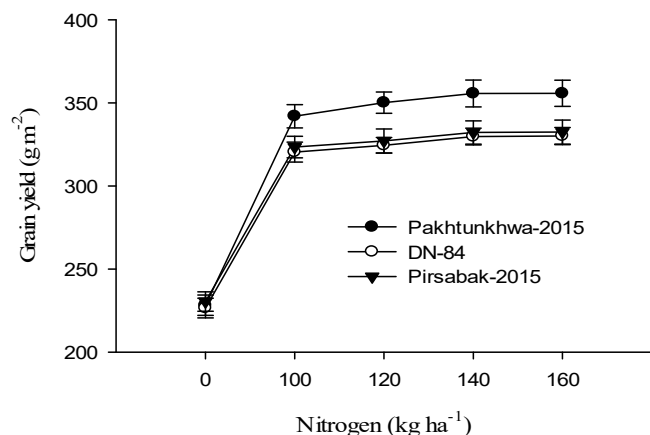


Figure 4. Grain yield ( $\text{g m}^{-2}$ ) of treatment interaction [N application rates (NAR) x wheat variety].

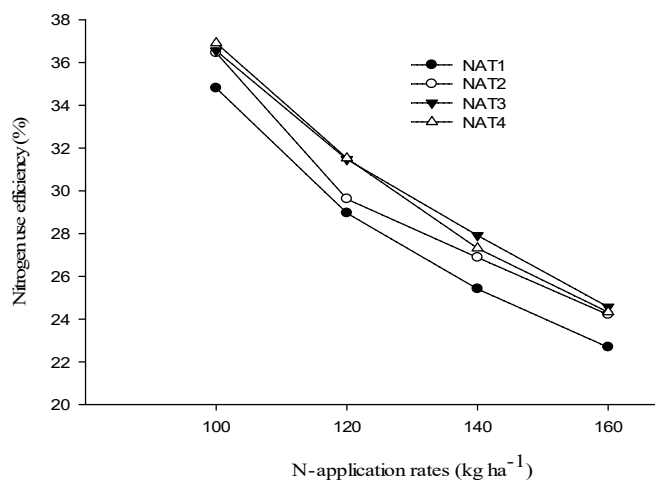


Figure 5. Nitrogen use efficiency (%) of treatment interactions [N-application rates (NAR) and N-application timings (NAT)].

**Crude protein (%):** Grain protein content (CP) as affected by NAR, NAT and varieties is shown in Table 2. While averaged across NAT and varieties, the NAR 140  $\text{kg ha}^{-1}$  showed higher CP which did not vary ( $p < 0.05$ ) from 160  $\text{kg N ha}^{-1}$ . CP did differ from 120  $\text{kg ha}^{-1}$ , followed by 100  $\text{kg ha}^{-1}$  with the lowest in control treatment. When averaged across NAR and varieties, the CP was highest in three splits (NAT<sub>3</sub>) with similar for NAT<sub>4</sub>. CP of grains was reported lowest in NAT<sub>1</sub> that did not differ from NAT<sub>2</sub>. Pirsabak-2015 showed higher

CP in grain, followed by Pakhtunkhwa-15 and lowest in DN-84. Interaction NAR x NAT was significant ( $p < 0.05$ ) with increasing trends for all NAT by increase NAR (Fig. 6). Difference in CP of varieties under NAT at any increment of NAR showed visible differences at NAR 120 and 160  $\text{kg ha}^{-1}$ . Both NAT<sub>3</sub> and NAT<sub>4</sub> performed better CP in grains at 140  $\text{kg ha}^{-1}$  as compared to NAT<sub>2</sub> or NAT<sub>1</sub>. The NAT and varieties showed a significant effect in grain CP (Fig. 7). Pirsabak-2015 was good, followed by DN-84 and Pakhtunkhwa-2015 at any given NAT with more clear differences for NAT<sub>3</sub> and NAT<sub>4</sub>.

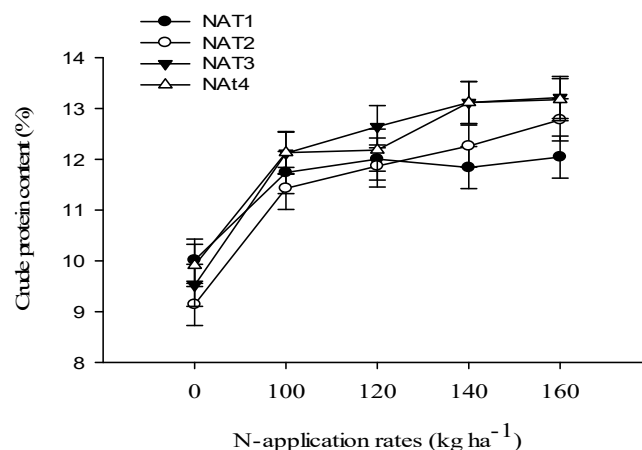


Figure 6. Crude protein (%) of treatment interactions [N-application rates (NAR) and N-application timings].

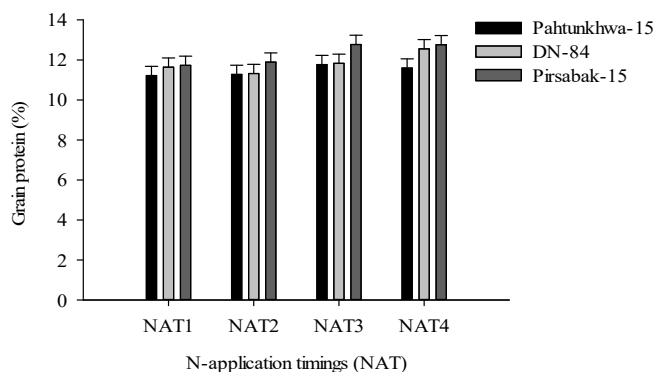


Figure 7. Crude protein content (%) of treatment interactions [N-application timings (NAT) and wheat varieties].

**Wet gluten ((%):** Wet gluten content (WGC) was significantly affected by NAR, NAT and varieties (Table 3). While averaged across NAT and varieties, the highest WGC was observed at NAR 140  $\text{kg ha}^{-1}$ , which was statistically similar to 160  $\text{kg ha}^{-1}$ . The WGC observed at 120  $\text{kg ha}^{-1}$  differed ( $p < 0.05$ ) from 100  $\text{kg ha}^{-1}$  with lowest in control. While mean across NAR and varieties, the highest WGC recorded at NAT<sub>4</sub>, followed by NAT<sub>3</sub> and NAT<sub>2</sub> with the

lowest in NAT<sub>1</sub>. Pirsabak-2015 showed higher WGC which was similar with DN-84 and lower in Pakhtunkhwa-2015. Treatment interaction NAR x NAT was found significant for WGC (Fig. 8).

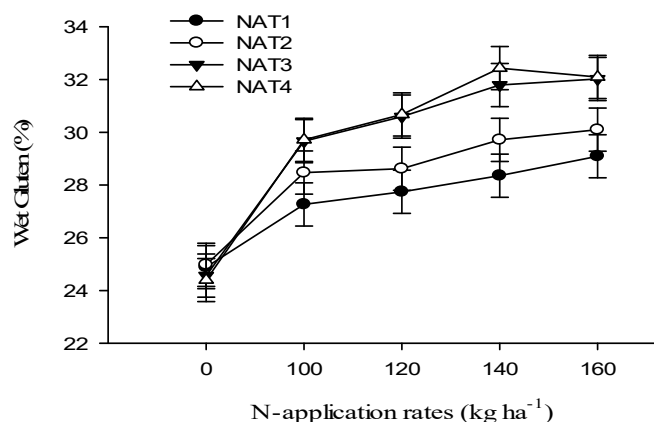


Figure 8. Wet gluten content (%) of treatments interaction [N-application rates (NAR) and N-application timing (NAT)].

Figure showed an increase in NAR from 0 kg N ha<sup>-1</sup> onwards showed an increasing trend for WGC with marked differences for NAT at NAR 100 approaching to 160 kg ha<sup>-1</sup>. Visible difference in WGC was observed under three splits when compared with two or single splits at 140 kg ha<sup>-1</sup>. Thereafter, WGC of wheat at NAR 120 and 160 kg ha<sup>-1</sup> did not show a clear response for any NAT.

**Dry gluten (%):** Dry gluten content (DGC) in grain as affected by NAR, NAT and varieties reported significant (Table 3). Averaged across NAT and varieties, the DGC was significantly affected by NAR with maximum for 140 kg ha<sup>-1</sup> with non-significant change from 160 kg ha<sup>-1</sup>, followed by 120 and 100 kg ha<sup>-1</sup>. The minimum DGC was noted in control. When averaged across NAR and V, highest DGC reported in three splits (NAT<sub>3</sub> and NAT<sub>4</sub>), followed by two splits (NAT<sub>2</sub>) and minimum by NAT<sub>1</sub>. The DGC in varieties did not differ ( $p < 0.05$ ). Interaction NAR x NAT showed change in DGC which were increased with increasing NAR in all NAT but with different readings at a given NAR for splits over sole applications. DGC at NAR 100 kg ha<sup>-1</sup> were higher for all NAT when compared with control. Thereafter, differences expanded for NAT at NAR 120 with highest at 140 kg ha<sup>-1</sup>

Table 3. Wet and dry gluten content (%) of wheat crop as influenced by N-application rates (NAR) and N-application timings (NAT).

N-application rates (kg ha <sup>-1</sup> ) NAR	Wet gluten (%)			Dry gluten (%)		
	2016-17	2017-18	Means	2016-17	2017-18	Means
0	26.4	25.5	26.0 d	8.5	8.7	8.6 d
100	30.1	27.5	28.8 c	10.9	11.0	10.9 c
120	30.3	28.6	29.4 b	11.1	11.2	11.1 b
140	31.4	29.7	30.6 a	11.4	11.3	11.3 a
160	31.8	29.8	30.8 a	11.5	11.4	11.4 a
LSD (0.05)			0.4			0.2
Nitrogen Timings (NAT)						
NAT <sub>1</sub>	28.0	26.9	27.4 d	10.4	10.5	10.4 b
NAT <sub>2</sub>	29.8	26.7	28.2 c	10.5	10.6	10.6 b
NAT <sub>3</sub>	31.0	28.7	29.9 b	10.8	10.9	10.9 a
NAT <sub>4</sub>	31.2	29.0	30.1 a	10.9	10.8	10.9 a
LSD (0.05)			0.4			0.2
Varieties						
Pakhtunkhwa-2015	29.7	27.8	28.8 b	10.7	10.7	10.7 a
DN-84	30.1	28.1	29.1 a	10.7	10.7	10.7 a
Pirsabak-2015	30.2	28.7	29.5 a	10.7	10.7	10.7 a
LSD (0.05)			0.3			Ns
Years means	30.0	28.2	**	10.7	10.7	Ns
Interactions						
NAR x NAT	Ns	ns	**	Ns	ns	**
NAR x V	**	ns	Ns	**	ns	Ns
NAT x V	Ns	ns	Ns	Ns	ns	Ns
NAR x NAT x V	*	ns	Ns	*	ns	Ns

NAT<sub>1</sub> = 100% at sowing; NAT<sub>2</sub> = 50% at sowing (0 DAS) & 50% at tillering (70 DAS); NAT<sub>3</sub> = 25% at sowing (0 DAS), 50% at tillering (70 DAS) & 25% at booting stage (100 DAS); NAT<sub>4</sub> = 25% at sowing (0 DAS), 25% at tillering (70 DAS) & 50% at booting stage (100 DAS). Means followed by different letter within a category of a treatments are statistically different from each other using least significant difference (LSD) test ( $p < 0.05$ ).

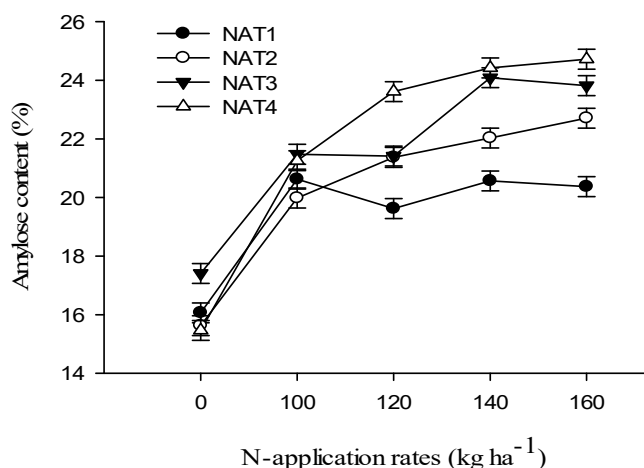
**Table 4. Amylose and amylopectin content (%) of wheat crop as influenced by N-application rates (NAR) and N-application timings (NAT).**

N-application rates (kg ha <sup>-1</sup> ) NAR	Amylose content (%)			Amylopectin (%)		
	2016-17	2017-18	Means	2016-17	2017-18	Means
0	18.2	17.3	17.7 d	83.3	84.4	83.9 a
100	21.8	21.6	21.7 c	79.4	78.9	79.2 b
120	23.5	22.1	22.8 b	77.9	79.1	78.5 b
140	23.8	23.8	23.8 a	77.2	77.2	77.2 c
160	23.9	24.0	23.9 a	77.2	77.0	77.1 c
LSD (0.05)			0.7			0.9
Nitrogen Timings (NAT)						
NAT <sub>1</sub>	20.6	20.1	20.3 c	80.7	81.3	81.0 a
NAT <sub>2</sub>	22.3	21.4	21.8 b	78.9	79.9	79.4 b
NAT <sub>3</sub>	22.9	23.2	23.0 a	78.5	77.9	78.2 c
NAT <sub>4</sub>	23.2	22.7	22.9 a	78.0	78.5	78.2 c
LSD (0.05)			0.6			0.8
Varieties						
Pakhtunkhwa-2015	22.2	21.8	22.0	79.1	79.4	79.3
DN-84	22.2	21.6	21.9	79.0	79.7	79.3
Pirsabak-2015	22.3	21.9	22.1	78.9	78.9	78.9
LSD (0.05)			Ns			Ns
Years means	22.2	21.8	Ns	79.0	79.3	Ns
Interactions						
NAR x NAT	ns	ns	**	Ns	ns	**
NAR x V	**	ns	Ns	**	ns	Ns
NAT x V	ns	ns	Ns	Ns	ns	Ns
NAR x NAT x V	*	ns	Ns	*	ns	Ns

NAT<sub>1</sub> = 100% at sowing; NAT<sub>2</sub> = 50% at sowing (0 DAS) & 50% at tillering (70 DAS); NAT<sub>3</sub> = 25% at sowing (0 DAS), 50% at tillering (70 DAS) & 25% at booting stage (100 DAS); NAT<sub>4</sub> = 25% at sowing (0 DAS), 25% at tillering (70 DAS) & 50% at booting stage (100 DAS). Means followed by different letter within a category of a treatments are statistically different from each other using least significant difference (LSD) test ( $p < 0.05$ ).

and narrow down at 160 kg ha<sup>-1</sup>. DGC was reported the highest with no changes for NAT<sub>3</sub> and NAT<sub>4</sub> at 140 kg ha<sup>-1</sup>, clearly lower value for NAT<sub>2</sub> followed by NAT<sub>1</sub>.

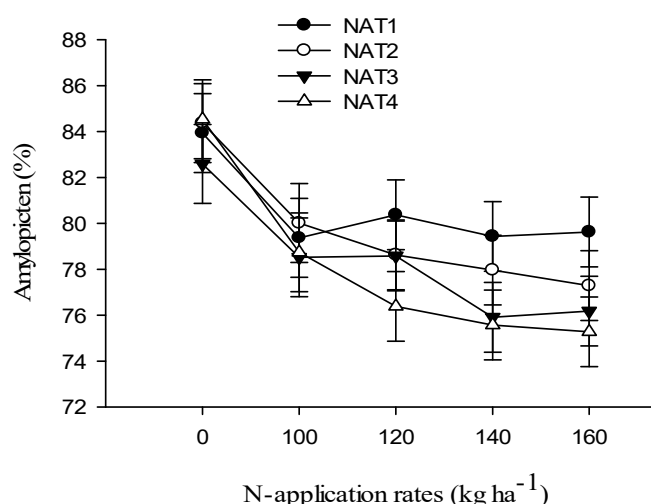
**Amylose (%):** Grain amylose content (GAC) as influenced by NAR, NAT and varieties is shown in Table 4. Statistical analysis of data revealed that NAR and NAT had significant effect on GAC with non-significant changes in varieties. Averaged across NAT and varieties, maximum GAC reported at NAR 140 kg ha<sup>-1</sup> which was non-significant ( $p < 0.05$ ) with 160 kg ha<sup>-1</sup>, followed by 120 and 100 kg ha<sup>-1</sup>. Lowest GAC was recorded in the control treatment. When averaged across NAR and varieties, the maximum GAC was observed in three splits (i.e., NAT<sub>4</sub> and NAT<sub>3</sub>), followed by two splits (NAT<sub>2</sub>) and lowest under the NAT<sub>1</sub>. For interaction, NAR x NAT showed significant effect in GAC (Fig. 9). As observed GAC showed increments with increasing NAR with different fashion particularly for splits application at any given NAR. The GAC showed almost same readings for NAT at control with slight changes at NAR 100. Thereafter, NAR increased from 100 to 120 kg ha<sup>-1</sup>, differences in GAC expands with highest at 140 and 160 kg ha<sup>-1</sup> where NAT<sub>4</sub> was markedly dominate NAT<sub>3</sub>, followed by NAT<sub>2</sub> and lowest by NAT<sub>1</sub>.



**Figure 9. Amylose content (%) for treatments interaction [N-application rates (NAR) x N-application timings].**

**Amylopectin (%):** Grain amylopectin (GAP) affected ( $p < 0.05$ ) by NAR, NAT and varieties (Table 4). Averaged

across NAT and varieties, NAR differed ( $p < 0.05$ ) in GAP, with highest in control, followed by 100 kg N ha<sup>-1</sup> which did not differ from 120 kg ha<sup>-1</sup>. The lowest GAP was observed NAR 140 kg ha<sup>-1</sup> which was similar with NAR 160 kg ha<sup>-1</sup>. Averaged across NAR and varieties, highest GAP was recorded in single application (NAT<sub>1</sub>), followed by two splits (NAT<sub>2</sub>) and subsequently three splits (NAT<sub>3</sub> and NAT<sub>4</sub>). Varieties were non-significant for GAP. Interaction (NAR x NAT) was significant for GAP with a decrease ( $p < 0.05$ ) by increasing NAR with highest for control, followed by 100 and lowest at 140 kg ha<sup>-1</sup> which did not differ from NAR 160 kg ha<sup>-1</sup> (Fig. 10). The GAP was almost same under NAR at control and markedly differs with highest for NAT<sub>1</sub>, followed by NAT<sub>2</sub> and NAT<sub>3</sub> with clear changes at NAR 140 and 160 kg ha<sup>-1</sup>.



**Figure 10. Amylopectin content (%) for treatments interaction [N-application rates (NAR) x N-application timings].**

## DISCUSSION

The efficiency of nutrients up take by the plant depends on various biotic and abiotic factors. Among the abiotic factors, rainfall is considered as one of the major factors influencing N loss and its availability to crop. Significant loss in yield is observed when rain occurs during critical growth stages of the crop. Especially, during anthesis where crop is more sensitive to abiotic stresses and cause severe reduction in yield and quality. Considering the strong relation of N leaching and volatilization with proper nitrogen management this study was designed to evaluate N-application rates and N-application timings for different wheat varieties. The present study was evaluated on spring wheat under NAR and NAT to observe the impact of N on grain yield and quality. Nitrogen application rate and timing have shown significant impacts on grain yield and grain N-content as compared to the control treatment (Ierna *et al.*, 2015). Higher grain yield and grain N

was observed with the application of 140 kg N ha<sup>-1</sup> given in three splits. Nitrogen fertilizer is a critical constituent of plants, its availability at critical growth stage (vegetative and reproductive) may also improve photosynthetic activity by improving chlorophyll content, hence better partitioning of assimilates to grains which increases final grain yield. Belete *et al.* (2018) also depicted similar results where they showed increasing N rate to a certain level in the wheat crop has improved the final yield. Nitrogen use efficiency was significantly decreased with each increment in NAR from 100 to 160 kg ha<sup>-1</sup>. Similar results were reported by (Caviglia and Sadras, 2001) that increment in nitrogen rate decreased nitrogen use efficiency. Split application is a useful strategy for increasing NUE and was observed higher in three splits application (NAT<sub>3</sub> and NAT<sub>4</sub>). Our results are in conformity with Jan *et al.* (2010) that split application has increased NUE. The genetic make-up of varieties also plays a key role in quality traits especially NUE (Wang *et al.*, 2011; Fageria *et al.*, 2005). Varieties might significantly differ for NUE because of its genetic potential (Haile *et al.*, 2012).

Nitrogen availability in soil is compulsory for better crop production (Cui *et al.*, 2008). Crude protein contents increased with N increments with maximum for higher NAR. Concurrently, treatment without N-application resulted in minimum crude protein. Grain protein content is considered as key factor for quality which is ensured by optimum N rates and proper application timing (Fageria *et al.*, 2005). Nitrogen is building blocks of protein and optimum crude protein is only possible in grain when there is proper availability of soil nitrogen during different stages of crop growth. For harvesting maximum grain crude protein, adequate amount of N at proper growth stage is important (Anthony *et al.*, 2003; Ooro *et al.*, 2011) stated that N-application rates and timings influence baking quality of wheat. In addition, they reported a significant improvement in grain protein with an increment in N-rates. Optimum N in conjunction with its proper application methods has maximized wheat productivity (Sohail *et al.*, 2013). Similarly, three splits of N-application resulted higher N-content and ultimately maximum grain protein. It was observed that for seed yield, crude protein, and NUE, split N fertilization was much more effective than applying total nitrogen or in two splits (Mohammed *et al.*, 2013). Grain protein content varies for varieties due to variation in uptake potential and genetic makeup (Kanu *et al.*, 2017). Variety Pirsabak-15 produced maximum protein content followed by DN-84 while, the minimum protein content was observed in Pakhtunkhwa-15.

The considerable variations in both growing seasons might cause changes in wet gluten content (Vrkoc *et al.*, 1995). Nitrogen application rate might affect wheat wet gluten content because nitrogen is building block of protein and essential component of plant growth and development (Qadeer *et al.*, 2019). Similar results were reported by Ottman *et al.* (2000) where increment in N-rate resulted in better grain

quality attributes, especially gluten. The split N-application is therefore essential for the optimum uptake and utilization of the plants and hence are responsible for better grain quality (Alcoz *et al.*, 1993). Several studies showed that N split application enhanced wheat protein concentrations and thereby reducing overall N-rate and limits N-losses in rhizosphere (Ayoub *et al.*, 1994). In the current study, variation among the gluten content for varieties might be due to the genetic potential of cultivars (Hafid *et al.*, 1996). Wheat dry gluten content varied significantly for both growing seasons. This variation in dry gluten of wheat over the season is because of the considerable variation in the weather parameters i.e. the distribution of rainfall in crop life cycle. Our results supported the published findings (Vrokok *et al.*, 1995; Garrido-Lestache *et al.*, 2005). They reported variations in gluten content through fluctuation in weather. Similarly, nitrogen application rate also affected dry gluten contents of wheat because nitrogen is one of the key nutrients and building block of the protein thereby responsible for plant growth and development (Qadeer *et al.*, 2019; Johansson *et al.*, 2004). Likewise, Ottman *et al.* (2000) reported that increment in N-rate result in better grain quality attributes especially dry gluten. The split N-application is, therefore, essential for the optimum uptake and utilization of plants and hence are responsible for better grain quality (Alcoz *et al.*, 1993; Xue *et al.*, 2016). Several studies showed that split application of the nitrogen enhance the protein concentrations of wheat and thereby reducing overall N-rate and limits N-losses in rhizosphere (Dinnes *et al.*, 2002).

Wheat starch composition is an important quality trait from the prospective of food technologists due to its importance in industrial applications (Sestili *et al.*, 2010). Currently, researchers are focusing on the production of starch with high amylose content because such starch offers high resistance to shear thinning if used as a thickening agent in other foods. The climatic factors affecting amylose contents of wheat is well documented by Buresova *et al.* (2010). Maximum amylose contents were observed for higher nitrogen rate application and decrease in N-rate from optimum decrease the starch formation in wheat (Singh *et al.*, 2011). Split application of nitrogen enhances the nitrogen uptake and physiological efficiency and is enhanced by the split application of nitrogen (Amiri *et al.*, 2014). Hence, results in formation of starches, with a higher amylose contents in wheat boosting grain quality (Maqsood *et al.*, 2013). The variation in the starch contents of wheat cultivars is due to the cultivar's capability to translocate the available nutrients to starches thereby enhancing final grain quality (Massaux *et al.*, 2008). Wheat grain comprised of various components. Of them amylopectin structure is of multiple clusters and each cluster includes various chains. These chains vary through length and position (Beroft *et al.*, 2012). Weather conditions affect grain compositions of wheat by manipulating the soil nutrients availability (Buresova *et al.*, 2010). Nitrogen

application at higher rate resulted in less amylopectin whereas decrease in N-rate improves the amylopectin contents (Wang *et al.*, 2008). Split application of nitrogen enhances the nitrogen uptake and physiological efficiency resulting in higher amylose contents and ultimately decreasing the amylopectin composition in grains (Gous *et al.*, 2015). The efficient N uptake by plants due to split N-application resulted in higher amylose and less amylopectin in wheat starches, boosting the grain quality (Xiong *et al.*, 2008). In conclusion, grain quality parameters i.e., grain N, protein content, amylose, wet and dry gluten were positively affected by 140 kg N ha<sup>-1</sup> under three splits (i.e. 25% N at sowing, 50% N at tillering and 25% N at booting stage). The three splits N application could be a useful strategy to overcome N losses, reduce cost of production and ensure grain quality in wheat crop as compared to the traditional two splits N application technique.

**Conclusions:** From the study, it is concluded that existing recommended two-splits N-application timings for wheat crop is no more effective under flood water irrigation system and subsequently changes expected to be observed in the rainfall pattern with climate change in Pakistan. The three splits application i.e. (NAT<sub>3</sub> or NAT<sub>4</sub>) at seedbed preparation, tillering and booting either 25, 50, 25 or 25, 25, 50 ratios, respectively is more effective to be applied as per recommended rate (i.e., 140 kg N ha<sup>-1</sup>) to improve grain yield and quality for good baking flour. Cultivars do play an important role in N-uptake, hence a recommended variety has to be selected for better N use efficiency for the cropping system.

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