Grain Yield, Nutrient Accumulation and Fertilizer Efficiency in Bread Wheat under Variable Nitrogen and Phosphorus Regimes

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Abstract: Balanced crop nutrition is one of the important tools necessary for sustaining crop productivity, maintaining soil fertility and ensuring food security worldwide. Nitrogen and phosphorus are essential plant macronutrients, nevertheless, most of the Pakistani soils exhibit moderate to severe deficiency of these nutrients. Furthermore, considerable genetic variations exist among wheat genotypes for nutrient acquisition and utilization which illustrate their differential nutrient requirements to produce higher yields. A field experiment was conducted in order to formulate balanced fertilizer recommendations for newly evolved wheat genotype SD-998 to achieve maximum yield potential. Ten treatments viz., 0-0 (control), 90-23, 90-45, 90-70, 120-30, 120-60, 120-90, 150-40, 150-75 and 150-110 kg N-P_2O_5 ha^{-1} were applied according to randomized complete block design with three replications. Analysis of variance revealed that growth, yield and yield related attributes as well as nutrient accumulation were significantly ($P \leq 0.05$) influenced by varying N and P rates. The maximum plant height (100.8 cm), number of tillers per plant (10), spike length (10.3 cm), number of grains per spike (66), 100-grain weight (4.10 g) and grain yield (4990 kg ha^{-1}) was recorded in treatment having 150-110 kg N-P_2O_5 ha^{-1}. The highest P recovery efficiency (20.8 %), agronomic N efficiency (19.4 kg kg^{-1}) and agronomic P efficiency (36.8 kg kg^{-1}) were attained at 120-30, 90-70 and 150-40 kg N-P_2O_5 ha^{-1}, respectively. However, the highest total N accumulation (140.8 kg ha^{-1}), P accumulation (21.0 kg ha^{-1}), N recovery efficiency (71.1 %) and profit (Rs. 73625 ha^{-1}) were noticed at 150 kg N plus 110 kg P_2O_5 ha^{-1}. Thus, 150-110 kg N-P_2O_5 ha^{-1} (N:P_2O_5 in 4:3 ratio) was suggested as the most balanced and economical dose for the tested wheat genotype.

Keywords: Crop nutrition, Fertilizer efficiency, Food security, Value-cost ratio, Yield.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important food grain crop for most of the population in Pakistan. It accounts for 1.9 percent of GDP and 9.6 percent of the value addition in agriculture and was cultivated on area of 9.05 million hectares with total production of 25.75 million tonnes during 2016-17 [1]. This average yield per unit area in Pakistan is low as compared to other wheat producing countries of the world [2]. Fertilizers are crucial inputs for enhancing wheat production, profitability and maintaining soil fertility [3]. Fertilizer use in Pakistan is continuously rising over the years but there is stagnation in crop yield largely due to imbalanced fertilizer usage. Farmers usually apply higher amounts of nitrogen but little or sub-optimal amounts of phosphorus and potassium resulting in negative soil nutrient balance [4].

Nitrogen (N) and phosphorus (P) are major essential nutrients for improving plant growth and crop yield by regulating an array of physiological, biochemical and molecular processes within plant body. Nitrogen is vital element for the synthesis of amino acids, enzymes, chlorophyll, vitamins, alkaloids and hormones [5, 6]. Its deficiency results in stunted growth, chlorotic leaves, reduced assimilate production, premature flowering and reduced growth period. While, excess of N increases chlorophyll formation and plant succulence leading towards reduced plant resistance to diseases and adverse climatic conditions [7]. Phosphorus is structural component of DNA, RNA, ATPs, phospholipids, enzymes and coenzymes and has central importance for the processes of photosynthesis, respiration, cell division/ enlargement and energy storage [8, 9]. It stimulates root growth and is associated with early maturity of crops. Phosphorus application balances the effect of excessive N by accelerating crop maturity and retarding excessive vegetative growth. Moreover, response of applied nitrogen in crops is also reduced under P deficiency [10].

Crop performance can be optimized by applying nutrients in balanced quantities on soils which are unable to supply these nutrients to meet crop demand. Balanced crop nutrition aims at a dynamic equilibrium between crop requirement and uptake of nutrients by crops and is a key to improve fertilizer use efficiency [11]. Judicious and balanced fertilization accelerates nutrient uptake, enhance grain yield, maintain soil nutrient balance and increase grower income [2]. Inter and intra-specific variations exist in crop plants for their nutritional requirements based on their genetic characteristics, plant architect and agro-climatic conditions of the region to produce maximum yield [4,

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12]. Several earlier studies revealed that various wheat genotypes have different fertilizer requirements to achieve maximum yield. In a field study, wheat cultivar TD-1 performed better at 120-60-60 kg N-P-K ha^{-1}, while Mehran-89 and T.J-83 showed maximum yield response at 180-60-60 kg N-P-K ha^{-1} [13]. Results of another study revealed that application of 175-150-125 kg N-P-K ha^{-1} was the optimum dose for wheat cultivar Sahar-2006 [14]. Likewise, application of N-P_2O_5 @ 120-90 kg ha^{-1} was found as best combination for the potential harvest of wheat genotype Khirman [15] and NIA-MB-2 [16]. In the same way, wheat variety NIA-Sunder was successfully grown with 150-110 kg N-P_2O_5 ha^{-1} for its potential yield [17]. A balanced fertilizer dose of 150-75 kg N-P_2O_5 ha^{-1} was recommended for wheat genotype BWQ-4 for achieving maximum economic harvest [18]. The present field experiment was therefore undertaken to evaluate wheat genotype SD-998 evolved at Nuclear Institute of Agriculture (NIA), Tando Jam for grain yield, nutrient accumulation and fertilizer efficiency under various nitrogen and phosphorus regimes.

MATERIALS AND METHODS

Plant Material and Crop Management

A field experiment was conducted during Rabi 2015-16 at NIA, Tando Jam, Pakistan to exploit the yield potential of newly evolved wheat genotype SD-998 under different fertilizer combinations. Seed of tested genotype was obtained from Plant Breeding and Genetics Division of NIA, Tando Jam. Soil samples from 0-15 cm surface layer were randomly collected from experimental site prior to wheat sowing and a composite soil sample was analyzed for selected soil physical and chemical characteristics using standard methods. Soil was clay loam in texture (22.50% sand, 41.34% silt and 36.16% clay) [19], alkaline in soil reaction with 7.8 pH_e [20] and 2.3 dS m^{-1} EC_e [21], 0.73% organic matter [22], 0.06% Kjeldahl nitrogen [23], 3.67 and 179 mg kg^{-1} AB-DTPA extractable phosphorus and potassium [24], respectively. Three nitrogen levels (90, 120 and 150 kg N ha^{-1}) and nine phosphorus levels (23, 30, 40, 45, 60, 70, 75, 90 and 110 kg P_2O_5 ha^{-1}) were combined in 4:1, 4:2, and 4:3 N:P_2O_5 ratios to formulate treatments along with control (without N and P). Treatments were applied by following the randomized complete block design (RCBD) with three replications.

Wheat crop was sown in each experimental unit of size 4m × 4m with row-row distance of 30 cm using single row hand drill. All treatments including control were fertilized with constant dose of potassium at the rate 60 kg K_2O ha^{-1}. Urea (46% N), di-ammonium phosphate (DAP, 46% P_2O_5 & 18% N) and sulphate of potash (SOP, 50% K_2O) were used as a source of N, P and K, respectively. The required quantities of P and K were applied at the time of seed bed preparation while N was applied in three equal splits viz., at sowing, tillering and booting stage. At crop maturity, data regarding yield and yield associated attributes, i.e. plant height, number of tillers per plant, spike length, number of grains per spike, grain yield, straw yield and 100-grain weight were recorded.

Nitrogen and Phosphorus Assay

The plant material (grain and straw) was oven-dried at 70 ^oC for 72 hours, followed by grinding using Thomas Wiley's mill (3383L10, Thomas Scientific, USA). Total nitrogen concentration in grain and straw samples was determined using fully automated digestion and distillation assembly (2200 Kjeltic, FOSS, UK) [23], while total P concentration was determined at 470 nm using a double beam spectrophotometer (U-2900UV/VIS, Hitachi, Japan) following standard protocol [25].

Nutrient accumulation, recovery efficiency and agronomic efficiency of N and P were calculated as follows [4]:

Nutrient Accumulation (NA)

NA (kg ha^{-1}) = [Yield (kg ha^{-1}) × Nutrient concentration in plant (%)] / 100

Nutrient Recovery Efficiency (NRE)

NRE (%) = [($NA_{treatment}$ – $NA_{control}$) / Nutrient applied] × 100

Agronomic Nutrient Efficiency (ANE)

ANE (kg kg^{-1}) = [(Grain yield $_{treatment}$ – Grain yield $_{control}$) / Nutrient applied]

Statistical Analysis

All the data obtained for growth, yield and yield related parameters, nutrient accumulation and nutrient efficiency relations were subjected to analysis of variance using computer based software STATISTIX 8.1 (Analytical Software, Inc., Tallahassee, FL, USA). The differences among treatment means were separated by least significant difference test at 5% probability level [26].

RESULTS

Growth, Yield and Yield Related Attributes

Application of nitrogen and phosphorus in different combinations significantly ($P \leq 0.05$) influenced the yield and yield related attributes of wheat. The data regarding yield parameters, i.e. plant height, number of tillers per plant, spike length, number of grains per spike and 100-grain weight are presented in Table **1**. Plant height increased linearly with corresponding increasing levels of P at each N level. The highest plant height (100.8 cm) was observed in plots receiving 150-110 kg N-P_2O_5 ha^{-1}. Number of tillers per plant varied with changing N and P rates. Plots fertilized with 120-90 and 150-110 kg N-P_2O_5 ha^{-1} showed maximum number of tillers per plant (10). Spike length was not influenced considerably and ranged from 7.4 cm in control to 10.3 cm in plots fertilized with 150 kg N plus 110 kg P_2O_5 ha^{-1}. Number of grains per spike (NGS) increased significantly with the addition of variable N and P rates. The maximum NGS (66) were recorded where N-P_2O_5 was applied at the rate of 150-110 kg ha^{-1} showing 34% increase over control. Likewise, minimum 100-grain weight (3.07 g) was observed in control while maximum (4.10 g) in plots having 150-110 kg N-P_2O_5 ha^{-1} which was statistically similar to 4.03 g obtained in plots receiving 120-90 kg N-P_2O_5 ha^{-1}. The data pertaining to grain and straw yield of wheat genotype SD-998 under various N and P regimes are given in Table **1**. Grain yield followed an increasing trend with subsequent addition of P at each N level. Numerically, control treatment produced minimum grain yield of 2397 kg ha^{-1} which was statistically different from all other treatments. The highest grin yield (4990 kg ha^{-1}) was obtained with 150-110 kg N-P_2O_5 ha^{-1} which was statistically at par to that obtained at 150-75 and 120-90 kg N-P_2O_5 ha^{-1}. Similar trend was also observed for straw yield. The highest straw yield of 7131 kg ha^{-1} was produced by plots receiving 110 kg P_2O_5 in combination with 150 kg N ha^{-1} showing an increment of 48% over control (3743 kg ha^{-1}).

Nutrient Accumulation (kg ha^{-1})

Nitrogen accumulation in grain and straw under different N and P levels is presented in Table **2**. Grain N accumulation was influenced significantly ($P \leq 0.05$) and its magnitude ranged from 26.5 kg ha^{-1} in control to 104.3 kg ha^{-1} (75% increase over control) with 150-110 kg N-P_2O_5 ha^{-1}. Straw N accumulation varied with additional P inputs at each N level. The highest straw and total N accumulation (36.6 and 140.8 kg ha^{-1}) was noticed at highest levels of N and P_2O_5, i.e. 150 and110 kg ha^{-1}. The highest P accumulation in grain (15.5 kg ha^{-1}) and straw (5.5 kg ha^{-1}) was recorded at 150 kg N and 110 kg P_2O_5 ha^{-1}. Total P accumulation (grain + straw) was recorded minimum (4.6 kg ha^{-1}) in control while its highest value (21.0 kg ka^{-1}) was noticed at 150-110 kg N-P_2O_5 ha^{-1} which was statistically different from all other treatments (Table **2**).

Nutrient Efficiency Relations

The data regarding nutrient efficiency relations, i.e. recovery efficiency and agronomic efficiency under

Table 1: Growth, Yield and Yield Related Attributes of Wheat Genotype SD-998 as Influenced by Various Rates of Nitrogen and Phosphorus

Treatments (N-P_2O_5 kg ha^{-1})	Plant height (cm)	No. of tillers $plant^{-1}$	Spike length (cm)	No. of grains $spike^{-1}$	100-grain weight (g)	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Biological yield (kg ha^{-1})
Control	73.6 g	5 c	7.4 b	43 d	3.07 g	2397 f	3743 g	6141 f
90-23 (4:1)	81.8 f	7 b	8.6 ab	49 d	3.41 f	3179 e	4417 f	7596 e
90-45 (4:2)	84.8 ef	8 ab	8.9 ab	51 cd	3.56 ef	3646 cde	5173 e	8819 d
90-70 (4:3)	87.3 de	9 ab	9.2 ab	58 abc	3.62 de	4146 bc	5731 d	9877 c
120-30 (4:1)	89.6 d	8 ab	8.9 ab	57 bc	3.50 ef	3458 de	4896 ef	8354 d
120-60 (4:2)	94.8 c	8 ab	9.4 a	61 ab	3.79 cd	4244 bc	5860 cd	10104 c
120-90 (4:3)	96.7 bc	10 a	10.1 a	63 ab	4.03 ab	4552 ab	6354 bc	10906 b
150-40 (4:1)	97.2 bc	8 b	9.6 a	58 abc	3.66 cde	3869 cd	5200 e	9069 d
150-75 (4:2)	99.3 ab	9 ab	10.0 a	61 ab	3.84 bc	4556 ab	6440 b	10996 b
150-110 (4:3)	100.8 a	10 a	10.3 a	66 a	4.10 a	4990 a	7131 a	12121 a
$LSD_{0.05}$	3.05	1.97	1.77	7.98	0.21	671	513	735

Treatment means sharing same letter(s) in the same column indicates non-significant differences.

Table 2: Nitrogen and Phosphorus Accumulation in Grain and Straw of Wheat Genotype SD-998 under Various Rates of Nitrogen and Phosphorus

Treatments ($N\text{-}P_2O_5$ kg ha^{-1})	Nitrogen accumulation (kg ha^{-1})			Phosphorus accumulation (kg ha^{-1})		
	Grain	Straw	Total	Grain	Straw	Total
Control	26.5f	7.7 f	34.2 h	3.3 f	1.3 f	4.6 g
90-23 (4:1)	39.2 e	15.2 e	54.5 g	7.1 e	2.2 e	9.3 f
90-45 (4:2)	47.8 de	19.7 de	67.5 f	9.1 d	2.6 de	11.7 e
90-70 (4:3)	54.8 cd	20.0 de	74.8 ef	11.2 bc	3.0 d	14.2 cd
120-30 (4:1)	60.5 c	22.5 d	83.0 f	8.1 de	2.7 de	10.8 ef
120-60 (4:2)	72.5 b	27.8 c	100.3 d	11.1 bc	3.7 c	14.8 c
120-90 (4:3)	81.8 b	27.8 c	109.6 cd	11.9 b	4.2 bc	16.1 bc
150-40 (4:1)	81.0 b	30.3 bc	111.3 c	9.4 cd	3.0 d	12.5 de
150-75 (4:2)	95.4 a	33.3 a	128.7 b	12.6 b	4.5 b	17.2 b
150-110 (4:3)	104.3 a	36.6 a	140.8 a	15.5 a	5.5 a	21.0 a
$LSD_{0.05}$	9.53	4.85	10.59	1.99	0.58	2.06

Treatment means sharing same letter(s) in the same column indicates non-significant differences.

Table 3: Nutrient Recovery, Agronomic Efficiency and Economic Analysis under Variable Rates of Nitrogen and Phosphorus

Treatments ($N\text{-}P_2O_5$ kg ha^{-1})	Recovery efficiency (%)		Agronomic efficiency (kg kg^{-1})		Economic analysis			
	Nitrogen	Phosphorus	Nitrogen	Phosphorus	Production value (Rs)*	Fertilizer cost (Rs)**	Profit (Rs)	VCR
Control	-	-	-	-	-	-	-	-
90-23 (4:1)	22.5 f	20.4 ab	8.7 c	34.0 ab	27668	9621	18047	2.88
90-45 (4:2)	37.0 e	15.8 de	13.9 abc	27.7 ab	46395	12472	33923	3.72
90-70 (4:3)	45.1 de	13.7 de	19.4 a	25.0 ab	64884	15712	49173	4.13
120-30 (4:1)	40.7 e	20.8 a	8.8 bc	35.4 ab	39038	12741	26297	3.06
120-60 (4:2)	55.0 bc	17.0 bcd	15.4 ab	30.8 ab	68629	16629	52000	4.13
120-90 (4:3)	62.8 ab	12.7 e	18.0 a	23.9 b	80965	20517	60448	3.95
150-40 (4:1)	51.4 cd	19.6 abc	9.8 bc	36.8 a	53252	16251	37001	3.28
150-75 (4:2)	63.0 ab	16.8 cd	14.4 abc	28.8 ab	81624	20786	60838	3.93
150-110 (4:3)	71.1 a	14.9 de	17.3 a	23.6 b	98947	25322	73625	3.91
$LSD_{0.05}$	9.75	3.48	6.64	12.19	-	-	-	-

Treatment means sharing same letter(s) in the same column indicates non-significant differences.
*Wheat grain + straw (wheat grain @ Rs. 1200 per 40 kg, straw/bhoosa @ Rs. 250 per 40 kg).
**Urea @ Rs. 1700 per bag, DAP @ Rs. 3650 per bag.

variable N and P regimes are given in Table **3**. Nitrogen recovery efficiency (NRE) was significantly ($P \leq 0.05$) influenced and depicted a linear increasing trend with corresponding P addition at each N level. The lowest NRE (22.5 %) was observed in plots receiving 90-23 kg $N\text{-}P_2O_5$ ha^{-1} while maximum (71.1 %) was recorded at 150-110 kg $N\text{-}P_2O_5$ ha^{-1}. Higher P recovery efficiency (PRE) was noticed with lower P rates at each N level. The highest PRE (20.8 %) was obtained in treatment receiving 120-30 kg $N\text{-}P_2O_5$ ha^{-1} which was statistically identical to 20.4 and 19.6 % obtained at 90-30 and 150-40 kg $N\text{-}P_2O_5$ ha^{-1}, respectively. Agronomic N efficiency (ANE) varied considerably under various N and P levels (Table **3**). Like NRE, the values of ANE were recorded maximum with high P levels at each N level. The maximum ANE (19.4 kg grain yield kg^{-1} of N applied) was recorded with 90 kg N + 70 kg P_2O_5 ha^{-1} and it was statistically at par to that obtained at 90-45, 150-75, 120-60, 150-110 and 120-90 kg N-P_2O_5 ha^{-1}. Agronomic P efficiency (APE) exhibited a decreasing trend with subsequent additional P inputs at each N level. The highest APE (36.8 kg kg^{-1} of P) was recorded at 150-40 kg N-P_2O_5 kg ha^{-1} while minimum (23.6 kg kg^{-1} of P) was observed in plots fertilized with 150 kg N plus 110 kg P_2O_5 ha^{-1}.

Economic Analysis

Net income generated and value cost ratios (VCR) were calculated in order to estimate profit gained by applying fertilizer inputs in various combinations (Table **3**). The minimum net production value (Rs. 27668) and profit (Rs. 18047) was obtained from plots receiving 90-23 kg N-P_2O_5 ha^{-1}, while maximum net production value (Rs. 98947) and profit (Rs. 73625) was recorded from plots fertilized with 150-110 kg N-P_2O_5 ha^{-1}. The highest VCR (4.13) was noticed in treatment having 90-70 kg N-P_2O_5 ha^{-1}, followed by 3.95, 3.93 and 3.91 obtained at 120-90, 150-75 and 150-110 kg N-P_2O_5 ha^{-1}, respectively.

DISCUSSION

The prime objective of the present study was to exploit the yield potential of newly evolved wheat genotype SD-998 under different fertilizer levels and to choose the most balanced and economical fertilizer combination. Fertilizers are crucial inputs for enhancing crop productivity, farm profitability and maintaining soil fertility [3]. Nitrogen and phosphorus are essential plant macronutrients, nevertheless, most of the Pakistani soils reveal moderate to severe deficiency of these nutrients [27, 28]. Therefore, application of fertilizers containing macro- nutrients is the recommended strategy to overcome native N and P deficiencies and to sustain crop production on such soils.

Application of various levels of N and P influenced significantly ($P \leq 0.05$) the yield and yield related attributes, i.e. number of tillers per plant, spike length, number of grains per spike. Plant height, tiller count and number of grains per spike increased linearly with corresponding increasing levels of P at each N level and were observed higher at 150-110 kg N-P_2O_5 ha^{-1}. Previous studies [29] have revealed that plant height increases considerably with the increasing rates of N and P_2O_5 in appropriate ratio. Moreover, synergistic effect among both N and P resulted in stimulated plant growth and increased absorption of these nutrients [30]. Our findings confirmed the fact that balanced and adequate availability of nutrients leads the crops to produce more fertile tillers. Similar outcomes have been reported earlier [2, 17]. Different researchers have reported substantial variations in spike length of wheat grown under different combinations of N and P combinations [2, 29]. Addition of nutrients in balanced ratio enhanced spikelets count per spike and finally number of grains per spike [12]. Increased grain weight with subsequent N and P fertilization might be due to enhanced chlorophyll content and photosynthetic rate ensuring plenty of photosynthates during grain filling [31].

Grain and straw yield of wheat genotype SD-998 was affected considerably by various N and P regimes but at variable rate. Grain and straw yield showed increasing trend with subsequent addition of P at each N level and maximum values were recorded at highest levels. Similar findings that each increment in P rate produced higher grain yields, have been reported earlier [32]. The better plant growth and enhanced straw yield with increasing N rates can be attributed to the most important function of N in enhancing vegetative growth [5]. On the other hand, adequate supply of N enhances protein content in both foliage and grains in cereals [33]. Similar trend in wheat grain yield by the addition of N and P_2O_5 has also been reported in previous experimentation [17]. Other researchers have also reported an additive effect of phosphorus on wheat performance if supplied with N in balanced proportion [34]. In present study, N and P accumulation by grain and straw was affected considerably under increasing P rates at each N level. Successive increment in P at each N level significantly enhances the N use efficiency reflecting strong synergism between both elements [15]. Balanced application of N and P has synergistic and stimulative effect on development of better root system which accelerates their uptake by plant [34].

Nutrient efficiency relations, i.e. recovery efficiency and agronomic efficiency were influenced significantly under variable N and P regimes. Recovery efficiency of any nutrient may be described as the amount of a particular nutrient in above ground plant parts per unit of applied nutrient, while agronomic efficiency (AE) is the economic production obtained per unit of nutrient applied [35]. Nitrogen recovery efficiency showed a linear trend with corresponding P addition at either N level. Other scientists have also disclosed the identical results [2, 17]. Recovery efficiency of N can be improved by the addition of P at a particular N level [36]. Phosphorus recovery efficiency and agronomic N efficiency were noticed higher with lower P rates at each N level. Recoveries of both nutrients can be improved by tightening the ratio between N and P suggesting that a reasonable merger of elements is important to enhance their use efficiency [16, 18]. Agronomic P efficiency was affected considerably and showed decreasing trend with subsequent additional P inputs at each N level. At each N level, value regarding AE of phosphorus decreased with successive addition of P. It was reported that that genotypes having higher value of AE also produce higher yields [37, 38].

Value cost ratio (VCR) is an excellent criterion for the economics of the commodity produced and determines the net profit for the grower. However, there is lack of guidance and production policies for farmers to make decision about their production plans with the exception of price index [18, 39]. The VCR varied greatly at different fertilizer levels and tend to decrease at higher levels of N and P. In present study, the highest VCR (4.13) was calculated in treatments having 90-70 and 120-60 kg N-P_2O_5 ha^{-1}, followed by 3.95, 3.93 and 3.91 observed at 120-90, 150-75 and 150-110 kg N-P_2O_5 ha^{-1}, respectively. This indicates that unnecessary application of higher fertilizer doses with no increase or marginal increase in yield is not cost effective.

CONCLUSION

The results of present study clearly indicated that wheat genotype SD-998 performed differentially under varying N and P fertilizer rates. The maximum grain yield and relative profit was achieved at fertilizer combination of 150 kg N plus 110 kg P_2O_5 ha^{-1} in the ratio of 4:3 (N:P). Hence, this combination of N and P can be suggested as the most balanced and economical dose for the tested wheat genotype.

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